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Impact of sire selection and breed on parasite resistance in sheep

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ABSTRACT

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Selection within and among breeds are strategies to mitigate the impact of parasitism given failing chemotherapeutics. While Texels have improved marketability compared to other parasite-resistant breeds, the mechanism by which Texels reduce fecal egg count (FEC) is unclear. The immune response to *Haemonchus contortus* (*Hc*) was compared in Texel, parasite-resistant St. Croix, and parasite-susceptible Suffolk sheep. Adult worms exposed to St. Croix- and Texel-derived peripheral blood mononuclear cells and serum *in vitro* had greater binding around the reproductive structures than Suffolk and worm egg release tended to be affected by breed ($P = 0.09$). Resistance in Texels may be caused by reduced worm fecundity. Genetic merit for parasite resistance can be predicted by FEC estimated breeding values (EBV). To validate the FEC EBV, Katahdin rams (n = 10) with High and Low FEC EBV were randomly mated to Katahdin ewes. Spring-born progeny were developed on pasture. Post-weaning, lambs (Year 1, n = 113; Year 2, n = 118) were dewormed, transported to the WVU Animal Science Farm, and infected with *Hc* for five weeks. At removal from pasture, FEC was lower in Low FEC-sired lambs ($P < 0.05$). When infected with *Hc*, a greater proportion of Low FEC-sired lambs had worm counts of zero ($P < 0.05$) and worm fecundity was lower ($P < 0.05$) compared to High FEC-sired lambs. Progeny FEC corresponds with sire FEC EBV. Low FEC-selected Katahdins may have an intermediary form of resistance where worm burden is limited and fecundity is reduced in worms that establish.

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TABLE OF CONTENTS

Abstract……..……..……ii

Acknowledgements……...…….…iii

Table of contents…….…....v

List of tables and figures……...….....viii

Chapter I: Literature review…….…1

Introduction……..……1

Parasite biology……..……..2

Host-parasite interactions: the immunologic response……....…….6

Texel parasite resistance……...…8

Katahdin parasite resistance……...…12

Evolutionally development of immunity……..…..15

Disease resistance…….…..17

Summary……21

Literature cited……...……23

Chapter II: Immune response to *Haemonchus contortus* in Texel sheep…….....…….35

Abstract…….…….35

Introduction……...…… 36

Materials and methods…….……...37

Results…….....……43

Discussion…….……...……..45

Literature cited……...…48

Figures……...….53

Chapter III: Effect of sire fecal egg count estimated breeding value on Katahdin lamb pasture performance…….....…57

Abstract……..……57

Introduction……58

Materials and methods……..…..60

Results……...…….63

Discussion…….……...……..67

Literature cited……...……69

Tables and Figures……..…73

Chapter IV: Effect of sire fecal egg count estimated breeding value on *Haemonchus contortus* infection in Katahdin sheep……...80

Abstract……..80

Introduction……81

Materials and methods……...….83

Results……...…87

Discussion…….……...……..…89

Literature cited……...……91

Tables and Figures……..…95

Chapter V: Discussion and future directions……..……..100

Introduction……..……100

Selection summary……...……100

Texel summary……..…101

Katahdin summary……...…105

Management alternatives……..……108

Literature Cited……...…….110

LIST OF TABLES AND FIGURES

Chapter II

Figure 1. Antibody binding to *Haemonchus contortus* egg and hatch rate……..53

Figure 2. *Haemonchus contortus* infection in Texel and Suffolk sheep……..…54

Figure 3. Cellular binding to adult *Haemonchus contortus in vitro* and subsequent egg release……..…..55

Figure 4. Proposed mechanisms for *Haemonchus contortus* resistance in St. Croix and Texel sheep compared to absence of resistance in Suffolk sheep…….….56

Chapter III

Table 1. Sire summary for Year 1 (YR1) and Year 2 (YR2) matings with estimated breeding values (EBV)……...…73

Table 2. Lambing summary……74

Table 3. Lamb performance summary with lamb estimated breeding value (EBV).……..75

Figure 1. Timeline of lamb management by year……..…..76

Figure 2. Year 2 fecal egg counts (FEC) and anthelmintic treatment……..……77

Figure 3. Lamb death losses and treatment…….……78

Figure 4. Lamb fecal egg count (FEC) estimated breeding value (EBV) and survivability.79

Chapter IV

Table 1. Sire summary for Year 1 (YR1) and Year 2 (YR2) with sire estimated breeding values (EBV)……..95

Figure 1. Challenge infection performance……96

Figure 2. Growth performance……...97

Figure 3. Adult worm counts and harvest fecal egg count (FEC)…….……..98

Figure 4. Relationships between worm count, fecal egg count (FEC) and post-weaning fecal egg count (PFEC) estimated breeding value (EBV)……...……..……..99

CHAPTER I: LITERATURE REVIEW

Introduction

Parasite infections are considered one of the greatest challenges facing small ruminant production globally. These parasite infections are frequently associated with the gastrointestinal nematode (GIN), *Haemonchus contortus* (*Hc*). Symptoms associated with acute Haemonchosis include reduced weight gain, wool growth, and reproductive fitness due to anemia and hyperproteinemia (Mavrot et al., 2015). Severe infections can result in death, leading to estimates of annual losses in the millions (Sackett et al., 2006). Compounding these losses is anthelmintic resistance in GIN populations, compromising long-term sustainability of small ruminant operations (Kaplan and Vidyashankar, 2012). Nonetheless, alternatives exist to combat these challenges through integrative parasite management strategies. These include pasture management through rotational grazing methods, utilizing tannin-containing forages, selective deworming through FAMAHCA™ scoring, nutritional supplementation and genetic selection (Maqbool et al., 2017). Genetic components of parasite resistance will be the focus of this review. Genetic resistance to GIN is variable within breed and across breeds (Gamble and Zajac, 1992; Notter et al., 2003; Vanimisetti et al., 2004b; Notter et al., 2007; NSIP Searchable Database, 2019). With moderate heritability, progress can be made through selection for sheep with greater parasite resistance (Vanimisetti et al., 2004a). Selection for parasite resistance is nearly requisite for sustainable small ruminant production in highly parasitized locales. Here, genetic resistance to GIN will be addressed in the context of parasite biology and management systems. Additionally, immune responses underpinning resistance will be highlighted with potential novel selection tools for general immunity.

Parasite Biology

The GIN of greatest concern to small ruminant production are considered members of the superfamily Trichostrongyloidea. These strongylid parasites include *Hc*, *Teladorsagia circumcincta* (*Tc*), and *Trichostrongylus* (Zajac, 2006). *T. circumcincta*, formerly known as *Ostertagia circumcincta*, larvae invade gastric glands of the abomasum (Levine, 1980). Consequently, abomasal HCl production is impaired and protein digestion is disrupted due to the inability to convert pepsinogen to pepsin, resulting in symptoms including hypoproteinemia, diarrhea and weight loss. This parasite is more common in temperate climates (Zajac, 2006). *Trichostrongylus* species can be found in the abomasum (*T. axei*) as well as the small intestine (*T. colubriformis* and *T. vitrinus*). Severe infections can result in diarrhea and weight loss (Bowman, 1999). While *Tc* and *Trichostrongylus* infections exist in the U.S., *Hc* is the primary cause of parasitic gastroenteritis in the United States and globally (Zajac, 2006).

Haemonchus contortus, another abomasal parasite also known as the Barber Pole Worm, is characterized by the appearance of female worms after feeding in which the blood-filled intestinal tract wraps around the uterus and reproductive structures of the adult worm. Larval and adult stages of *Hc* use a swimming motion to maintain their position in the abomasum. The lancet structure on the head of the worm punctures the epithelium resulting in blood release. Adult *Hc* can consume as much as 0.05 ml of blood per day resulting in anemia, hypoproteinemia, and death in severe infections (Taylor et al., 2007). In subclinical infections, reduced weight gain, milk production and wool growth may be observed (Mavrot et al., 2015). This worm is highly prolific with a single female releasing thousands of eggs each day. These eggs are released into the environment via fecal matter where they hatch into the L1 stage larvae and continue through two additional molts to the L3 or infective stage. This stage migrates to

forage where they reside until consumed by the host. Once consumed, larvae go through an additional molt to the L4 stage and then develop to adult worms. The period from infection to egg release by adult worms (prepatent period) is 17 to 21 days (Levine, 1980). A similar life cycle is observed with nearly all Trichostrongylid parasites.

Estimated economic losses from *Hc* are in the millions (Sackett et al., 2006) and *Hc* preferentially impacts areas with necessary climatic conditions. This parasite prefers warm and wet environments making the Southeastern U.S. very favorable for larval development (Craig, 1986). Strongylid larval development is optimal at temperatures between 10° and 36° C (Levine, 1980). Even so, the parasite is able to arrest development when unfavorable environmental conditions exist. This arrested development, or hypobiosis, typically occurs at the L4 stage and allows larvae to survive for extended periods of time in the host until a more favorable environment exists (Eysker, 1997). Adult worms live for only a few months in the host, so hypobiosis provides a means to extend or delay the parasite life cycle through harsh winter months (Levine, 1980). In the northern hemisphere, spring cues signal arrested larvae to continue development to an adult stage, because favorable environmental conditions exist for larvae to develop. These cues are thought to be related to a suppression in host immunity that occurs around the time of parturition or related to changes in endocrine hormone concentrations near lambing (Gutiérrez-Amézquita et al., 2017). Consequently, a rise in adult worm burden around the time of lambing is frequently associated with a rise in fecal egg count (FEC) over the period closely following parturition. This rise in FEC is referred to as the periparturient rise (PPR, Gibbs, 1986). This PPR typically peaks around day 30 post-parturition and is higher in ewes rearing multiple lambs (Notter et al., 2017). This peak coincides with lambs beginning to graze and allows for reinfection of young, naive individuals that are more susceptible to the parasite

(Zajac, 2006). As a result, lamb FEC rise peaks at around 120 days of age. Additionally, lambs reared as multiples typically have greater egg counts (Notter et al., 2017). There is also a genetic component contributing to the PPR and a relationship between PPR and lamb FEC. Heritability estimates for PPR at parturition and 30 days post-parturition were 0.35 and 0.24, respectively. The PPR at 30 days post-parturition had a 0.19 phenotypic correlation with lamb weaning and post-weaning FEC (Notter et al., 2018). In summary, hypobiosis and the subsequent PPR allow the strongylid life cycle to continue into a new growing season and infection of lambs permits continued propagation of parasitic worms in an immunocompromised host.

Given the global impact of strongylid parasites, specifically *Hc*, and associated pathologies, effective treatment and management are critical for the sustainability of small ruminant production. According to the 2011 USDA NAHMS survey, 89% of operations used an oral anthelmintic treatment in the previous year. Of these operations, approximately 80% utilized anthelmintic treatment as a general preventative. Approximately 35% used treatment because clinical signs were observed and only 11% used FAMACHA scoring to assess anemia in their flocks (NAHMS, 2011). Clearly, parasitic gastroenteritis is of concern to sheep producers; however, judicious use of anthelmintics needs to be addressed as anthelmintic administration occurred primarily as a preventative rather than treatment. With each anthelmintic treatment, selection pressure is placed on worms that are resistant to the anthelmintic. Given the prolificacy of these parasites, propagation of resistant genetics is rapid. Consequently, a rise in anthelmintic resistance has been observed in GIN populations (Fleming et al., 2006). In a study of small ruminant farms in the Southeastern United States, anthelmintic resistance to all three classes of anthelmintics was observed on 48% of farms. Specifically, resistance to thiabendazole, levamisole and ivermectin was observed on 98%, 54% and 76% of operations, respectively

(Howell et al., 2009). These three classes (benzimidazoles, macrocyclic lactones and cholinergic agonists) have specific and unique modes of action and if resistance to one class is developed in a worm population, typically all anthelmintic products within that class are ineffective. The benzimidazoles inhibit microtubule (β-tubulin) development in the parasite whereas the macrocyclic lactones impair chloride channel neurotransmission and the cholinergic agonists are considered paralytics (Bowman, 1999). Because worm movement is essential for maintenance of position within the abomasum, any restriction in motion results in larval expulsion through smooth muscle contractions of the gastrointestinal tract.

These three classes of anthelmintics have been the only classes available for the last 30 years (Kaplan and Vidyashankar, 2012). With the likely restricted development or approval of new anthelmintics, alternatives must be considered for parasite management. These alternatives are most effective when utilized in an integrative parasite management plan in which multiple management tools are put into place in one system. As outlined by the American Consortium for Small Ruminant Parasite Control, alternatives to anthelmintics include genetic selection, nutritional management through supplementation and grazing tannin-containing forages, selective deworming using FAMACHA scoring and administration of copper oxide wire particles.

Increases in protein nutrition can improve host ability to respond to parasitic infection. This can be done through use of feedstuffs with greater protein content or, more specifically, incorporation of a feedstuff high in rumen bypass protein such as fishmeal. Tannins bind protein at high pH but release that protein when pH drops. Therefore, utilization of tannin-containing forages increases rumen bypass protein in an individual's diet (Hoste et al., 2016). All of these alternatives should be considered in a management plan. However, for the purposes of this

review, the focus will be placed on genetic selection for more resistant individuals. This resistance can be considered in terms of breed differences in resistance to GIN or within breed differences if great variability exists within one breed of sheep (Woolaston et al., 1990; Notter et al., 2007; NSIP Searchable Database, 2019).

Host-Parasite Interactions: The Immunologic Response

The mechanism by which the host controls and eliminates a parasite infection is mediated through host immune responses. In sheep, these responses have been characterized using parasite-resistant compared to parasite-susceptible breeds or by studying responses in divergently selected lines. Historically, St. Croix hair sheep have been used to model parasite resistance (Gamble and Zajac, 1992; Notter et al., 2003; Bowdridge et al., 2013). In general, this breed inhibits adult worm establishment and is the standard for true resistance (Jacobs et al., 2015).

Compared to wool breeds [Dorset, Suffolk, or Dorset X Rambouillet X Finnsheep (WFX)], the St. Croix has reduced FEC during both primary and challenge infections with reduced adult worm burdens (Gamble and Zajac, 1992; Notter et al., 2003; Jacobs et al., 2015). This reduction results from a multifaceted immunological response which is established earlier in resistant vs. susceptible breeds (Bowdridge et al., 2013; Bowdridge et al., 2015). Reduced worm burden within the first seven days of infection was accompanied by increased white blood cell counts and increased IgA in St. Croix compared to WFX sheep. Globule leukocytes, eosinophils and neutrophils increased in both breeds following infection. However, neutrophil concentration was greater in the St. Croix compared to the Suffolk at three, five, and seven days after infection (Bowdridge et al., 2015). Even so, the St. Croix is better able to generate antigen-specific IgA maintaining a lower FEC during the periparturient period (Bowdridge et al., 2013). The ability of

St. Croix to recognize *Hc* antigen and promote an early response may be critical to preventing adult worm establishment. An enhanced humoral and cellular response has been documented to the L3 larval stage compared to both the exsheathed-stage L3 as well as the L4 stages (Garza et al., 2017; Garza et al., 2018). Thus, recognition of the L3 stage is paramount. Once exsheathed, these subsequent larval stages have an improved ability to evade host immunity. *In vitro*, exsheathment can occur within 12 hours with L4 larvae developing by 48 hours (Garza et al., 2018).

In addition to cellular recruitment to the site of infection, T-helper cell type 2 (Th2) polarization has been associated with GIN infection (Kooyman et al., 2000; Balic et al., 2002; Pernthaner et al., 2005; Lacroux et al., 2006) and with increased IL-4, IL-5, IL-13 production. MacKinnon et al. (2015) characterized this polarization in the St. Croix compared to the WFX. Here, increased expression of IgE, IL-5 and IL-13 was observed in the lymph nodes of the St. Croix by day 3 post-infection, with increased IgE and IL-13 expression in the abomasal tissue by day 27. In this comparison with WFX, IL-4 differences were not observed. However, when compared to Suffolk sheep, St. Croix had increased IL-4 expression within the first seven days of infection (Jacobs et al., 2016) and were able to maintain higher IL-4 production through day 49 post-infection (Jacobs et al., 2015). In addition to expression in epithelial tissue, peripheral blood mononuclear cells (PBMC) stimulated with larval antigen increased their expression of IL-4Ra, IL-13, IL-5 and MRC1 in cells from St. Croix compared to those of Suffolk sheep (Jacobs et al., 2018). Clearly, the St. Croix is able to generate a Th2 type immune response to *Hc*. Consequently, larval expulsion results due to increased smooth muscle contractility and mucus release from goblet cells (McKenzie et al., 1998; Herbert et al., 2009) and little adult establishment is seen.

In contrast, increased expression of IL-12 p35 and IFN-γ in the WFX compared to the St. Croix indicate Th1 polarization rather than a Th2 phenotype (MacKinnon et al., 2015). Further evidence of an impaired Th2 response is seen in greater expression of IL-17 in PBMC from the Suffolk when stimulated with larval antigen (Jacobs et al., 2018). Here, a Th1 phenotype is observed with greater evidence of immune regulation. The combination of delayed recognition of infection and inappropriate Th1/Th2 polarization results in an environment more favorable for larval establishment in these wool breeds.

In summary, resistance to helminth parasites is classically associated with Th2 polarization. This polarization is characterized by increased IL-4, -5 and -13 production resulting in increased IgE, smooth muscle contractility and an increase in mucus production leading to larval expulsion. Consequently, adult worm establishment is inhibited and no FEC is observed. The failure to generate this response allows adult worm establishment.

Texel Parasite Resistance

Texel sheep were introduced to the United States in 1985. They were first imported from Finland and Denmark to the U.S. Meat Animal Research Center (Clay Center, NE) and after initial research on terminal sire utilization was completed, the breed was made available to U.S. sheep producers (Leymaster and Jenkins, 1993). Historically, the Texel breed has been considered a terminal sire breed with exceptional muscle development. Studies in the U.K. have demonstrated increased dressing percentage (Latif and Owen, 1980; Wolf et al., 1980; Ellis et al., 1997) and cutability with larger loin muscle areas than Suffolk sheep (O'Ferrall and Timon, 1977; Wolf et al., 1980). Studies in the U.S. have observed similar results. While the Suffolk breed has growth advantages (Ali et al., 2005; Mousel et al., 2012; Notter et al., 2012), the Texel

excels in leg scores and carcass conformation (Leymaster and Jenkins, 1993; Mousel et al., 2012; Shackelford et al., 2012). Even so, performance and fitness of progeny in forage-based production systems may be necessary given certain production systems, especially those systems in the Eastern U.S. Parasitism associated with forage-based sheep production is the largest challenge facing producers. Therefore, parasite resistant phenotypes in terminal sire breeds could be beneficial in producing market-acceptable lambs with fitness attributes for forage-based production systems.

To address this question, Good et al. (2006) and Sayers et al. (2008) observed differences in FEC between Texel and Suffolk sheep. At three sampling ages (11, 14 and 17 weeks of age), Suffolk lambs consistently had greater FEC than Texel lambs. This was observed in a research flock as well as in producer flocks. Adult worm counts in the abomasum were higher in the Suffolk as well (Good et al., 2006). Since then, numerous papers have been published addressing proposed mechanisms for such resistance. In general, these studies have focused on genetic markers associated with mechanisms related to helminth immunity.

IFN-γ is commonly associated with a Th1 response. This inflammatory response is typically observed in microbial infections, but early IFN-γ may be observed in parasite resistant sheep (Coltman et al., 2001). Sayers et al. (2005) examined haplotype differences between the Texel and the Suffolk in intron 1 of the IFN-γ gene. While all haplotypes (A, B, C, and D) were present at similar frequencies in the Suffolk, only haplotypes A and B were present in the Texel, with the A haplotype occurring in 79% of the population. In the Texel, the B haplotype was associated with a reduction in FEC. In the Suffolk it was not. Possible transcription and translation of intron 1 in the Texel could result in an IFN-γ dysfunction. In the Suffolk, proper

removal of intron 1 results in normal IFN-γ production (Sayers et al., 2005). Consequently, the reduced IFN-γ in the Texel could result in improved Th2 polarization and GIN resistance.

In addition to the IFN-γ gene, there has been considerable attention paid to major histocompatibility complex (MHC) haplotypes and their association with resistant phenotypes. DRB1 region (MHC II) has been a focus for nematode resistance. In particular, the DRB1*1101 allele has been of interest. This allele has been associated with nematode resistance in Scottish Blackface sheep (Schwaiger et al., 1995) as well as reduced FEC in the Suffolk breed (Sayers et al., 2005). Even so, little association has been seen with this allele and resistance in the Texel breed (Sayers et al., 2005). Ali et al. (2019) examined the relationship between MHC haplotypes as well as IgA and IgE activity more closely in the Texel. Seven of the 15 haplotypes were associated with FEC and one of these, the 11b haplotype, was associated with the DRB1*1101 allele. However, this haplotype also was associated with reduced IgA activity. Even so, a lower worm burden could result in a reduced IgA response. Still, the mechanism of GIN resistance in the Texel breed is unclear.

While a greater understanding of genetic markers for resistance could provide selection tools, the ability of a host to resist infection and reduce FEC is rooted in the immunological response to the parasite. While this response has a genetic component, the immune mechanism provides a greater understanding of host/parasite interactions. As observed by Good et al. (2006), the maintenance of FEC in Texel sheep despite lower averages than the susceptible Suffolk suggest the persistence of some adult worm burden. Therefore, adult worm mediated immunity must be examined. Stear et al. (1999) hypothesized that lambs can control worm length but not number and as lambs mature to adults, worm burden can be regulated. These data were based on heritabilities of worm number and length in Scottish Blackface sheep. Further relationships have

been shown between worm length and fecundity. As worm length increases, egg release increases. The regression coefficient associated with this relationship is 1.03 or a ten-fold increase in worm number is associated with a ten-fold increase in FEC (Stear et al., 1995). Even still, as worm number increases in the abomasum, worm length has been shown to decline (Stear et al., 1999). Additionally, there has been considerable attention given to the role of IgA in helminth resistance. While some studies have related worm length to IgA concentrations, indicating that increased IgA results in shorter worms (Smith et al., 1985; Stear et al., 1995), others have seen very little response in IgA to adult worm antigens (Schallig et al., 1995). In a direct comparison of Suffolk and Texel sheep, Sayers et al. (2008) saw a divergence in IgA, IgG, and IgE beginning at 11 weeks of age. Texel sheep had greater antibody concentrations than the Suffolk. On the contrary, Ahmed et al. (2015) observed no differences between the breeds for serum or mucosal IgA. Even so, higher pepsinogen concentrations have been noted in Suffolk compared to the Texel (Sayers et al., 2008; Ahmed et al., 2015). While considerable evidence exists associating increased IgA concentrations with decreased worm length and fecundity, further evidence is needed to confirm IgA-mediated immunity in the Texel. All said, increased FEC, worm burdens and pepsinogen levels in the Suffolk indicate greater GIN susceptibility and pathogenicity when compared to the Texel breed.

Evidence of reduced worm burden and improved parasite resistance in the Texel indicates a unique opportunity for the Texel as a parasite-resistant terminal sire. Even so, a majority of the work done up to this point has been associated with general *Trichostrongyle* egg counts or specifically *Tc* as this GIN is more prevalent in the temperate climates of Europe. Additionally, the mechanism of resistance in the Texel is still uncertain. A better understanding of the immune

response particularly to *Hc* would be beneficial to U.S. producers looking to utilize the Texel as a terminal sire in forage-based production systems.

Katahdin Parasite Resistance

The Katahdin was developed by Michael Piel of Maine as an easy-care meat breed with a shedding hair coat. As a composite, this breed was developed from a variety of genetic sources. To incorporate a hair phenotype, "African Hair Sheep" were imported from the island of St. Croix in 1957. These were then crossed with traditional wool breeds such as the Suffolk, Hampshire, Southdown and Tunis to improve conformation. After 15 years of selection, the name "Katahdin" was given to the new breed after the tallest mountain in Maine (KHSI Breed Origin & History, 2019). Since that time, the Katahdin breed has grown to become the most frequently registered breed in the United States (Morgan, 2019). Their forage-adaptability and perceived "parasite resistant" phenotype has led to their increased popularity especially in the Southeastern United States. In the last 20 years, hair sheep as a percentage of the U.S. sheep flock have risen from less than 1% to 26%. In the Southeast alone, the number of farms raising sheep has increased by 167% (Newton, 2019). Clearly, the influence of hair sheep and the Katahdin breed has been pronounced.

While the breed has been branded as "parasite resistant," significant variability exists for parasite resistance (Notter et al., 2007; NSIP Searchable Database, 2019). Traditionally, FEC has been used as the metric to assess parasite resistance or susceptibility. Beginning in 2003, the National Sheep Improvement Program (NSIP) began collecting FEC data to generate estimated breeding values (EBV) for parasite resistance. These EBVs are expressed as a percentage and reflect the individual's genetic merit for changing FEC (Notter and Lewis, 2018). These FEC

EBVs are generated for weaning FEC (WFEC, 45-90 days of age) and post-weaning FEC (PFEC, 90 to 304 days of age) and currently range from -100 to 596 and -100 to 1800, respectively (NSIP Searchable Database, 2019). Individuals with lower FEC EBVs are expected to be more parasite resistant. Even with these selection tools, the mechanism underlying these differences in FEC within a single breed has not been fully elucidated.

In studying the genetic basis of the Katahdin breed, parasite resistance traits likely originated from the original "African Hair Sheep" that founded the breed. While the exact origin or breed of these "African Hair Sheep" is unclear, similar genetic ancestry of St. Croix hair sheep could be assumed based on geographic proximity. The Virgin Island White is likely the common ancestor between the St. Croix and the Katahdin. The Virgin Island White existed on the island of St. Croix and was the basis of the St. Croix breed. The St. Croix, however, was not brought to the mainland U.S. until 1975, approximately 15 years after the development of the Katahdin began (Wildeus, 1997). When comparing the genetic structure of U.S. sheep breeds, the Katahdin is most similar to African hair breeds such as the St. Croix and Barbados Blackbelly (Blackburn et al., 2011).

Evidence exists for parasite life-stage dependent immunity. Larval stage mediated immunity is observed in St. Croix sheep where adult worm establishment is inhibited and FEC is close to zero (Jacobs et al., 2015). In contrast, Texel sheep have a persistent, yet reduced FEC compared to the susceptible Suffolk (Good et al., 2006) indicating the maintenance of some adult worm population. Stear et al. (1995) proposed an IgA-mediated reduction in worm length and fecundity in Scottish Blackface sheep. It is plausible that a similar mechanism exists in the Texel. Thus, an adult worm burden is sustained but reductions in FEC are observed in individuals assumed to be more resistant. The Katahdin is a derivative of the St Croix, so it could

be hypothesized that more parasite resistant, low FEC EBV Katahdin sheep have increased genetic relatedness to the St. Croix and a similar mechanism of GIN resistance. Even so, establishment of an adult worm infection would indicate an imperfect St. Croix mechanism and opens the door for adult-stage mediated immunity through the reduction in worm fecundity.

Nevertheless, through utilization of EBVs in the Katahdin breed, a thorough understanding of genetic variability in GIN resistance has been established. Heritability estimates for FEC in the Katahdin range from 0.18 for WFEC to 0.23 for PFEC (Ngere et al., 2018). These estimates are similar to those seen in WFX where h^2 in lambs was 0.10 to 0.19 and in ewes was 0.31 (Vanimisetti et al., 2004a). These FEC heritabilities are similar to those seen for weaning and post-weaning body weights (Ngere et al., 2017) indicating similar opportunities for genetic progress. Weaning and post-weaning FEC have genetic and phenotypic correlations of 0.82 and 0.29, respectively (Ngere et al., 2018). Despite not having a clear understanding of the immunological mechanism, there is a genetic component to parasitism in the Katahdin breed and selection opportunities exist within breed. In comparison with other breeds, the Katahdin has lower FEC than the Dorper (Burke and Miller, 2002; Vanimisetti et al., 2004b). While both breeds have a hair phenotype, the Dorper is native of South Africa with a genetic make-up of Dorset Horn and East African genetics. The Caribbean hair sheep ancestry of the Katahdin make the breed more comparable to the St. Croix and Barbados Blackbelly, which have a West African ancestry (Spangler et al., 2017).

Selection for reduced FEC and improved parasite resistance has been demonstrated in Australian Merino flocks (Woolaston et al., 1990). In these flocks, selection for reduced FEC also reduced the PPR in selected low FEC ewes (Woolaston, 1992). Selection for improved parasite resistance in the Katahdin based on the FEC EBV is certainly expected. However, the

mechanism has not been elucidated. Genetic similarities exist with both the St. Croix and Barbados Blackbelly; however, greater FEC in the Katahdin compared to St. Croix X Blackbelly crossbred lambs indicate an imperfect mechanism in the Katahdin (Vanimisetti et al., 2004b; Blackburn et al., 2011). Even so, significant variability exists within the Katahdin breed for FEC, and greater investigation is needed to understand mechanisms driving variability.

Evolutionary Development of Immunity

Variation among breeds in immunologic mechanisms underpinning parasite resistance may indicate environmental-dependent development of immunity. Development of an adaptive immune response to the parasite is essential for long-term resistance. Müller et al. (2018) suggested that development of the adaptive immune system through somatic diversity and clonal selection is based on both quantitative and diversified exposure to antigens. Consequently, variability in the type of antigen exposure along with duration and intensity of exposure may influence the type of adaptive response the host develops. From this, the origin of immunologic mechanisms underpinning different responses observed among breeds to *Hc* could be hypothesized.

To better understand this mechanistic variability, differential antigen exposure and quantitative antigen exposure should be examined independently. First, differences in quantitative antigen exposure among breeds could have impacted their evolutionary development of immunity. Based on environmental differences, the tropical climate of St. Croix's origin would have resulted in greater parasite exposure compared to the more temperate climate of the Texel's origin. Based on this hypothesis, increased exposure to the parasite by St. Croix hair sheep during their evolutionary development could explain their larval stage-oriented response as

opposed to the Texel. In the St. Croix scenario, lack of larval-stage recognition could quickly result in excessive infection, associated pathologies and likely host death. Therefore, immune responses in St. Croix sheep are mediated by larval-stage recognition due to natural selection. However, in the Texel, larval exposure and development of adult worm infections may occur more slowly. The Texel was developed in north-central Europe where the temperate climate may have resulted in lower larval burden when compared to a tropical, Caribbean environment. Thus, failure to recognize the larval stage may not compromise host survival. In the Texel scenario, greater time may have existed for the host to recognize and respond to adult and egg stages of the parasite. Consequently, immunological orientation may have shifted towards the developing adult worm infection.

Another hypothesis for the observed mechanistic differences could be associated with the differential antigen exposure between breeds. As previously elucidated with the Texel scenario, time of exposure and immunological response needed may influence specific parasite life-stages recognized. Antigenic components vary between larval and adult stages of *Hc* (Riffkin et al., 1996). Infective larvae have a protective cuticle which is shed upon entering the abomasum. This cuticle can modulate the effectiveness of host immune response (Garza et al., 2017). Additional structural variation between larval and adult stages include development of reproductive structures. Differential recognition of these components implies differences in antigenic specificity between host immune responses. In the St. Croix, the necessity for a rapid response may have resulted in selection toward individuals that can recognize larval antigens and elicit an immune response. In the Texel, a shorter period of larval exposure may have resulted in a response oriented towards adult worm fecundity as worm populations slowly grew over time and

the necessity of a humoral and cellular response was recognized by increased abomasal epithelial damage from worm feeding.

In summary, the combination of differential and quantitative antigen exposure differences between breeds/evolutionary environment may explain observed mechanistic differences in parasite-resistant breeds. Evolutionary development in these individuals' specific responses may be rooted in somatic diversity passed from generation to generation (heritable) and clonal selection, which allows for adaptability to a variety of antigenic environments. This type of evolutionary development can be described as Darwinian immunity (Müller et al., 2018).

Disease Resistance

In 2011, the NAHMS reported death losses due to internal parasites on 15.5% of sheep operations accounting for 10% of sheep lost. Even so, additional losses from respiratory diseases, enterotoxemia and other digestive problems were reported on 12.8%, 4.2% and 7.8% of operations respectively accounting for 6.1%, 2.1% and 4.2% of sheep lost. In addition, ewe and lamb morbidity can result in reduced performance and treatment costs. Related to morbidity, 24.6%, 24.4% and 30.6% of operations reported treating ewes for respiratory disease, lameness and mastitis, respectively (NAHMS, 2011). Genetic selection for resistance to parasitism has been discussed previously. Tools such as EBVs exist to facilitate selection decisions and FEC provide a relatively simple metric to evaluate parasite burdens. Measuring resistance and fitness traits to these other diseases can become much more difficult (Snowder, 2006). Thus, an easy to measure metric which is associated with these traits could be a beneficial selection tool for improving disease resistance, individual fitness and possibly general immunity.

In these regards, the population of Soay sheep on Hirta Island in the St. Kilda archipelago has been used to study wild population dynamics related to parasitism and the influence of humoral and cellular immunity on individual fitness related to survival through "crash" winters. These "crash" winters are characterized by the loss of at least half the population (Walter, 2006; Hayward et al., 2011). Parasite burdens are characterized primarily by *Teladorsagia* species and *T. axei* (Craig et al., 2006). In "non-crash" years, lambs with greater FEC had reduced annual survival rates. However, in "crash" years, there was no relationship between FEC and survival (Hayward et al., 2011). Lamb IgA concentration was correlated negatively with FEC (Coltman et al., 2001). However, larval (*Tc*) antigen specific IgA had no relationship with overwinter survival. Even so, there was a positive relationship between antigen specific IgG and overwinter survival (Watson et al., 2016; Sparks et al., 2018). This relationship was independent of age, sex, FEC and body weight and there were no cellular markers associated with survival (Watson et al., 2016). This finding supports previous findings from Nussey et al. (2014), who also found positive associations between survival and total IgM and anti-nuclear antibody (ANA) concentrations independent of age and weight. These ANA are classically associated with cell replication and antibody production (Lipsky, 2001) and can be utilized as markers of autoimmunity (Lleo et al., 2010). Antinuclear antibody levels in these Soay sheep have a genetic basis with a heritability of approximately 13%. This relationship between increased ANAs and overwinter survival is apparent only in "crash" winters. Even so, females with elevated ANAs produced lambs that had greater survivability through the neonatal period (Graham et al., 2010). This wild population provides an interesting perspective on parasitism, immunity and fitness traits. Graham et al. (2011) suggested that maximum host fitness is not necessarily defined by the lowest parasite burdens or the greatest immune responses. While FEC was correlated negatively

with survival in normal years, the opportunity or ability to generate antigen specific antibodies indicated greater fitness in “crash” winters. Some parasite burden may be required for optimum fitness. Further, ANAs may be associated with improved fitness in sheep but also have been associated with autoimmune diseases such as systemic lupus erythematosus in humans if elevated titers are sustained (Arbuckle et al., 2003; Smee et al., 2007). An immune response is necessary for control of parasitism and disease fitness but even here, an extreme phenotype may be deleterious.

In domestic populations, selection for disease resistance and improved fitness could diminish the need for drug treatments and lessen chances of antimicrobial resistance and treatment costs (Stear et al., 2001). However, selection for resistance can result in increased sampling and diagnostic expenses associated with phenotyping (Snowder, 2006). It is estimated that economic losses from bovine respiratory disease (BRD) are close to $14/hd in feedlots (Snowder et al., 2006). Considerable work has been done in cattle as well as swine examining genetic components of these economically relevant diseases. Porcine reproductive and respiratory syndrome (PRRS) as well as BRD have moderate heritability (0.18 for BRD; Snowder et al., 2006; 0.17 to 0.45 for PRRS; Serão et al., 2014; Putz et al., 2018; Hess et al., 2018). Estimates of BRD heritability were based on BRD incidence in feedlot calves at US MARC. PRRS estimates were based on sample-to-positive (S/P) ratios of anti-PRRS IgG ELISA tests. While genetic progress through selection can reduce incidence of these diseases, time and sample costs could be cost-prohibitive (Snowder, 2006). Substantial benefits could be realized if the genetic component controlling resistance to multiple diseases shared common genes or similar loci. In such case, selection for resistance to one disease could be used for selection of general rather than specific immunity. Thus, the benefit from investments in sample collection

and analysis could be maximized. In the case of parasitism, selection for reduced FEC could be used for selection of resistance to not only parasite infection but also to other pathogens. Cattle with reduced parasite burdens on feedlot arrival had 16% lower treatment rates and a reduction in treatment costs of $27/hd (Clark et al., 2015). Improvements in feedlot health could be realized through reductions in parasite burdens.

Co-heritability can be used to describe a shared genetic component between two traits. It can be expressed as $r_g h_1 h_2$ where r_g is the genetic correlation between the traits and h_1 and h_2 are the square roots of the heritabilities. Co-heritability estimates were positive for internal parasite resistance compared to both footrot and dermatophilosis in Merino sheep (Raadsma et al., 1997). Even so, these relationships were low indicating differences in the majority of genes controlling resistance to these diseases. Beyond data from the Camden Merino flock, little evidence exists supporting broad resistance and general immunity to a variety of diseases in sheep. In general, these disease resistance traits are polygenic with many genes contributing to the observed phenotype. Additionally, it appears as though these genes are largely independent for each disease. Heritability estimates for disease resistance range from 0.05 to 0.80 for diseases such as internal parasites, footrot, flystrike, dermatophilosis and fleece rot (Raadsma et al., 1998). To better understand immunological fitness, antibody responses to vaccination for clostridial diseases as well as *D. nodosus*, known to be associated with footrot, were examined. Co-heritability for antibody titers of *D. nodosus* serotypes A-G to *C. tetani* as well as to *C. chauvoei* ranged from 0.12 to 0.41 (Raadsma et al., 1996). Therefore, some genes associated with humoral immunity and development of antigen specific antibodies to these vaccines are homologous. Even so, the degree to which these genes are shared varies by antigen combination. Nonetheless, genetic evidence exists to indicate a relationship between resistance to multiple diseases or

antigens. Given this evidence, selection for resistance to one disease may be associated with some level of resistance towards another disease. However, the relationship is likely small.

In summary, ewe and lamb morbidity and mortality result in economic losses from increased treatments, reduced performance and death. Improvement of individual fitness and disease resistance through selection could be a means to mitigate these losses in the midst of concerns for anthelmintic and antibiotic resistance (Raadsma et al., 1998; Stear et al., 2001; Snowder, 2006). Improvement in parasite resistance based on selection for reduced FEC is an example of this. In this case, FEC is an accurate and relatively easy to measure indicator trait for resistance. Quantitatively measuring disease status for other causes of morbidity can be more difficult. Even so, there appears to be a genetic component to susceptibility/resistance to a number of diseases across species (Snowder et al., 2006; Serão et al., 2014; Putz et al., 2018; Hess et al., 2018). In the Soay sheep population, relationships have been found between parasite-specific antibody levels and fitness traits (Watson et al., 2016; Sparks et al., 2018). Additionally, coheritability was positive between parasite resistance and footrot/dermatophilosis (Raadsma et al., 1997). If resistance to internal parasites is related to improved general fitness and resistance to other pathogens, FEC could be a valuable metric to select for disease resistance.

Summary

Parasitism poses a significant threat to the sustainability of small ruminant production globally. Widespread resistance to anthelmintic treatments has resulted in the development of alternative management strategies to mitigate the chances of parasite exposure and infection (Kaplan and Vidyashankar, 2012; Maqbool et al., 2017). The summation of these management strategies can be described as integrated parasite management plans.

One of these strategies includes genetic selection for individuals more resistant to infection. This resistance can be viewed as within or among breeds. Historically, parasite resistance was described in certain breeds particularly those of Caribbean or tropical origin. The St. Croix and Barbados Blackbelly serve as standard parasite-resistant breeds. These breeds limit adult worm establishment through larval-stage recognition and consequently have little to no FEC (Jacobs et al., 2015). Even so, other breeds such as the Texel, with origins in a more temperate climate, have now been described as having improved resistance (Good et al., 2006). However, a persistent yet reduced FEC compared to susceptible breeds shows some level of adult worm infection, which indicates a unique mechanism of resistance independent of those breeds of tropical origin.

Nonetheless, great variability exists within a single breed for resistance to parasitism. This has been best understood in divergently selected Merino flocks in Australia as well as the Katahdin breed in the U.S. (Woolaston et al., 1990; Notter et al., 2007; NSIP Searchable Database, 2019). Utilization of FEC EBVs has allowed quantification of parasite resistance and accurate selection to ensue. Even so, the composite nature of the Katahdin breed has resulted in great diversity within the breed. An open herdbook and vague records of original matings make the genetic composition of the Katahdin unclear and highly variable. Consequently, the mechanism underpinning resistance in low FEC Katahdins remains uncertain.

Selection for disease resistance is challenging due to the quantification of disease status and appropriate disease challenge (Snowder, 2006). Fecal egg counts provide a predictive measure of parasitism and are relatively easy to collect and measure on a continuous scale. For other diseases such as footrot and respiratory or clostridial diseases, measurement is not as simple. Predictive measures of susceptibility to these other pathogens could be very useful in

selection schemes and management programs. If resistance to parasitism could be used as an indicator of general immunity and environmental fitness, then the FEC EBV may be useful in a broad range of management settings. However, evidence is limiting to make a genetic connection between resistance to parasitism and resistance to other disease (Raadsma et al., 1997; Raadsma et al., 1998). Even so, parasite-specific IgG levels have been associated with survivability and fitness in wild Soay sheep (Watson et al., 2016; Sparks et al., 2018). Sheep able to generate antibody titers to the parasite also appear to have improved survival over winters on Hirta Island. This could possibly suggest an improvement in general immunity and lamb fitness.

Production challenges imposed by GIN can result in substantial economic losses from reduced animal performance and death losses (Sackett et al., 2006). Nonetheless, opportunities exist to improve the sustainability of small ruminant production through genetic selection for improved GIN resistance. Improved resistance can mitigate the need for anthelmintic treatment, thus, limiting selection of anthelmintic resistant GIN and maintaining a better refugia population (Muchiut et al., 2018). Even so, immunological mechanisms underpinning selection for improved resistance to parasitism are not fully understood for Texel as well as Katahdin sheep. These commercially relevant breeds serve U.S. sheep industry needs through terminal sire and maternal genetics, respectively. Improved management and selection practices could be incorporated in integrated parasite control measures given a better understanding of these mechanisms.

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CHAPTER II: IMMUNE RESPONSE TO *HAEMONCHUS CONTORTUS* IN TEXEL SHEEP

Abstract

Immune response of parasite-resistant St. Croix sheep specifically targets larval stages of *Haemonchus contortus* (*Hc*) preventing establishment and making this breed a standard for parasite resistance. While Texel sheep have low fecal egg counts (FEC) similar to St. Croix, adult worm counts are similar to susceptible breeds like Suffolk. Thus, the objective of this study was to compare immune responses of St. Croix, Texel and Suffolk sheep to adult- and egg-stage *Hc*. *Haemonchus contortus* adult worms and eggs were exposed to peripheral blood mononuclear cells (PBMC) and serum from St. Croix, Texel and Suffolk sheep *in vitro*. Fluorescently labeled anti-sheep IgA and IgG were used to measure antibody binding and quantified by ImageJ™ using corrected total egg fluorescence. Cellular binding to adult worms was observed using bright field and scanning electron microscopy. Egg release per worm was quantified. There was greater IgA binding to eggs when treated with St. Croix and Texel serum ($P < 0.05$) and these eggs exhibited a lower hatch rate ($P < 0.05$) when exposed to serum and PBMC. Adult worms exposed to St. Croix and Texel-derived PBMC and serum had greater binding around the head, genital pore and bursa than worms exposed to Suffolk-derived cells and serum. Consequently, egg release over 24 hours tended to be affected by breed ($P = 0.09$). To further examine differences, Suffolk and Texel lambs (n = 5/breed) were infected with 10,000 *Hc* L3 and harvested 30 days after infection. Worm counts and FEC were determined at harvest. Regressing FEC on worm count revealed that Suffolk sheep had greater egg release per worm than Texel (2.89 vs. 1.61 eggs/worm, respectively). Taken together, these data indicate a unique resistance

mechanism in Texel sheep targeting adult and egg-stage *Hc* reducing worm fecundity and egg survival.

Introduction

Immune response to the gastrointestinal nematode (GIN) *Haemonchus contortus* (*Hc*) in parasite-resistant St. Croix sheep specifically targets the larval stage, preventing adult worm establishment. More rapid, robust T-helper type 2 (Th2) cellular and humoral immune responses of St. Croix sheep mediate larval expulsion (Bowdridge et al., 2013; Bowdridge et al., 2015; Jacobs et al., 2015; Jacobs et al., 2016). Consequently, adult worm establishment does not occur and FEC in St. Croix sheep is near zero (Jacobs et al., 2015). In parasite-susceptible Suffolk sheep, recognition of *Hc* infection is delayed (Bowdridge et al., 2015; Jacobs et al., 2018) permitting adult worm establishment and subsequent fecal egg output. This model of *Hc* resistance or susceptibility is characterized by the direct relationship between adult worm presence and FEC.

Texel sheep were imported to the United States from the Netherlands in 1985 for their superior carcass characteristics (Leymaster and Jenkins, 1993). Good et al. (2006) reported reduced fecal egg count (FEC) in Texel sheep compared to Suffolk in the U.K., where *Teladorsagia circumcincta* infections are more common in these temperate climates. Evaluation of these breeds in U.S. hair sheep crosses observed improved muscling in the Texel with similar parasite resistance traits to the purebred hair lambs (Weaver, 2017). Major histocompatibility complex II (MHCII) haplotypes, including the DRB1*1101 allele, have been associated with parasite resistance in Scottish Blackface (Schwaiger et al., 1995), Suffolk (Sayers et al., 2005)

and Texel sheep (Ali et al., 2019). Even so, the immunological mechanism underpinning this resistance and specific responses to *Hc* in Texel sheep have not been elucidated.

The St. Croix was developed in parasite-favorable conditions of the Caribbean Islands (Wildeus, 1997). Thus, survival of St. Croix sheep was likely dependent on a resistant phenotype due to the persistent exposure to GIN larval stages. A more temperate climate of north-central Europe may have influenced evolution of parasite resistance in Texel through different mechanisms. Decreased acute larval exposure and consequent larval recognition may have resulted in a gradual increase in adult worm burden over time with minimal lamb mortality. Nonetheless, FEC of Texel sheep is reduced compared to more susceptible breeds (Good et al., 2006).

Therefore, the immunological mechanism unpinning FEC reduction observed in St. Croix and Texel sheep should be compared. A greater understanding of immunological targets in the Texel would allow for better implementation of the breed into terminal sire breeding schemes. Improved parasite resistance and moderate mature size make the Texel a potential option for sheep producers looking to add market acceptability to their flocks in parasite-burdened grazing environments.

Materials and Methods

Worm Egg Isolation

Fecal samples were collected from Suffolk wethers persistently infected with *Hc* housed in a dry lot environment preventing reinfection. Feces were diluted in water and centrifuged at 260 *x g* for 10 min at room temperature (RT). Supernatant was removed and feces were reconstituted in Sheather's solution (SPG 1.20 g/mL). Samples were centrifuged for 5 min at

3000 *x g* at 4° C. The top 1-mL containing suspended eggs was removed and washed 3 times in water to remove excess Sheather's solution. Egg suspension was then layered on lymphocyte separation medium (LSM, SPG 1.077 g/mL; Corning, Manassas, VA, USA) and centrifuged for 30 min at 900 *x g* at 4° C. The layer containing suspended eggs was removed and washed with water twice. Purified eggs were counted in 100 μL and total content was extrapolated. Egg suspensions were diluted in phosphate buffered saline (PBS, pH 7.4; Corning, Manassas, VA, USA) for binding and hatch assays.

Antibody Binding Assay

Antibody binding to worm eggs was quantified by breed (St. Croix, Suffolk, or Texel). Blood was collected from St. Croix, Suffolk and Texel mature ewes (n = 5/breed) via jugular venipuncture into 10-mL red-top vacutainer tubes (BD, New Jersey, USA) with no additive. Blood was allowed to clot for 30 min at RT and then centrifuged at 1500 *x g* for 20 min. Serum was transferred to 1.5-mL microcentrifuge tubes and stored at -80° C until use. Serum was added at 10% total volume to 500 eggs diluted in 250 μL of PBS in triplicate. All 1.5-mL microcentrifuge tubes and 96-well microplates (Corning, Corning, NY, USA) were blocked with 1% bovine serum albumin (BSA) in PBS prior to adding treatments. Eggs were allowed to incubate at 37° C and 5% CO_2 with serum for 1 hr. Following incubation, tubes with eggs were centrifuged (250 *x g* for 3 min at RT). Eggs were washed twice with 0.1% BSA diluted in PBS.

Anti-sheep IgG (Sigma Aldrich, St. Louis, MO, USA) and IgA (Bio-Rad, Hercules, CA, USA) were fluorescently labeled using ReadiLink Antibody Labeling kit 594/610 (Bio-Rad, Hercules, CA, USA). Antibody was diluted in PBS to 1 mg/mL concentration, then 5 μL of reaction buffer was added to 50 μL of antibody solution. Solution (55 μL) was added to one vial

of labeling dye and incubated for 1 hr at RT, after which 5 μL of quencher was added to reaction mixture and incubated for 10 min at RT.

Fluorescently labeled anti-sheep IgG or IgA (1:100 dilution in 0.1% BSA in PBS) were added to eggs and allowed to incubate for one hour at 37° C and 5% CO_2. This solution was centrifuged (250 *x g* for 3 min) and washed twice with 0.1% BSA PBS. The egg pellet was suspended in 400 μL PBS and 100 μL was added to each of three wells of 96-well microplates (Corning, Corning, NY, USA) for microscopy. Florescence was measured at 594 nm excitation and 620 nm emission on a Zeiss Axio Vert.A1 inverted microscope (Zeiss, Oberkochen, Germany). AxioCam ERc 5s microscope camera mounted on the Vert.A1 was used for micrographs. Exposure time was 523.6 ms and Image J (National Institutes of Health, Bethesda, MD, USA) was used to trace and measure fluorescence on 5 randomly selected eggs for each breed and replicate. Binding was quantified using corrected total egg fluorescence where measured fluorescence was adjusted for background illumination.

Peripheral Blood Mononuclear Cell (PBMC) Isolation

Spring-born St. Croix, Suffolk, and Texel lambs, previously exposed to *Hc* challenge infection but not currently infected, were used as donors for PBMC isolation. Blood was collected by jugular venipuncture into 10-mL EDTA-treated vacutainer tubes (Tyco, Mansfield, MA, USA). Blood was centrifuged at 1000 *x g* for 20 min at RT. The buffy coat was removed, resuspended in PBS and excess red blood cells were lysed using ACK (Lonza, Walkersville, MD, USA) for 5 min at RT. To stop the lysis, PBS was added and suspension centrifuged at 100 *x g* for 5 min at RT. The solution was reconstituted with PBS and layered on LSM. This suspension was centrifuged at 400 *x g* for 20 min. The middle layer containing PMBC was

removed and suspended in PBS then centrifuged at 100 *x g* for 5 mins. Supernatant was removed and pellet was resuspended in PBS. Cells were enumerated using Bio-Rad TC20 Automated Cell Counter. Cells were diluted to 1 X 10^6/mL in complete media containing RPMI-1640 (GE Healthcare Life Sciences, Logan, UT, USA) with 10% fetal bovine serum (FBS; Corning, Corning, NY) and 1% penicillin-streptomycin antibiotic (Sigma Aldrich, St. Louis, MO).

Egg Hatch Assay

To determine the effects of PBMC and serum on worm egg viability, serum and cells were incubated with worm eggs for 24 hr and hatch rate was determined. Fifty *Hc* eggs were added to each well (n = 3 replicates/breed) of a 96-well plate. Serum was added at 10% total well volume (150 µL). One million PBMC were added to each well. The plate was incubated at 37° C and 5% CO_2. Egg hatch was determined based on the proportion of larvae present in each well compared to the total number of eggs placed in each well.

Animal Infection and Worm Quantification

All animal procedures were approved by the West Virginia University Animal Care and Use Committee (protocol# 1608003811.1). Suffolk and Texel (n = 5/breed) lambs were orally inoculated with 10,000 *Hc* L3 diluted in PBS. Following infection, FEC and packed cell volume (PCV) were measured weekly. Fecal egg count was quantified using the modified McMaster's test and egg count was multiplied by 50 for extrapolation to eggs/g (Whitlock, 1948). The infection was allowed to persist for 30 days. Sheep were harvested at the Virginia Tech Meat Center. Abomasum contents were flushed with water and stored with 10% formalin (Thermo Scientific, Waltham, MA, USA) for quantification of worm burden. Palpable lymph nodes, in the

lesser curvature of the abomasum, were removed, counted and weighed. Contents were sorted and adult worms removed from debris. Total adult worm count (WC) was determined. One hundred worms were randomly selected from each lamb sample and mounted on 75 x 35 x 1 glass slides (VWR International, Radnor, PA, USA) with lactophenol (VWR International, Radnor, PA, USA). Worms were imaged using AxioCam ERc 5s microscope camera mounted on VistaVision stereomicroscope (0.65x); length was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA) and sex was determined.

Adult Worm Collection

A naïve Suffolk wether was infected with 10,000 *Hc* L3 and the infection was allowed to persist for 30 days for development of adult worms. The sheep was euthanized and the abomasum removed. Abomasum contents were emptied, adult worms were removed and placed in warm (37° C) PBS with penicillin-streptomycin antibiotic (Sigma Aldrich, St. Louis, MO). A series of washes with the same solution was used to clean worms of abomasal debris. At all times, adult worms were kept on a warming plate (37° C, Chicago Surgical and Electrical Co, Melrose Park, IL, USA). Male and female worms were sorted and placed one worm per well in 24-well plates (Corning, Manassas, VA, USA). Serum from St. Croix, Suffolk, and Texel ewes were added to respective wells (10% total volume). One million PBMC were added to each well from each respective breed. Cultures were allowed to incubate at 37° C and 5% CO_2 for 24 hr. Following incubation, bright field microscopy was used to observe cellular binding to adult worms. Worms bound by PBMC of each breed were selected for scanning electron microscopy (SEM).

Scanning Electron Microscopy

Worms were fixed in 2.5% glutaraldehyde for 1 hr. After washing three times in PBS for 15 min, worms were exposed to 2% OsO_4 for 1 hr. Wash was repeated three times. Worms were dehydrated in 15 min increments using 30%, 50%, 70%, and 90% ethanol. Worms were exposed to 100% ethanol three times for 15 min each time. Worm samples were air dried for 12 hr. Electron microscopy was performed by West Virginia University Shared Research Facilities.

Statistical Analysis

The Mixed Model procedure of SAS (SAS Institute, Cary, NC) was utilized to evaluate FEC and PCV differences with fixed effects of breed, time and breed X time interaction. Fecal egg count data were natural log transformed for normality. All other data were analyzed using fixed effects of breed. Worm sex and breed X sex interaction were included for WC data.

Worm fecundity was predicted based on FEC prior to harvest and WC by breed and analyzed using the General Linear Model procedure of SAS. The linear regression model was:

$$Y_{FEC} = \beta_1 WC + \beta_2 Trmt + (\beta_1 WC * \beta_2 Trmt) + e_{FEC}$$

where $\beta_1 WC$ represents the WC at harvest, $\beta_2 Trmt$ represents breed, $(\beta_1 WC * \beta_2 Trmt)$ represents the interaction of WC and breed, and e_{FEC} represents the random error associated with the prediction of FEC.

Significance was determined at $P \leq 0.05$. Tendencies were determined at $0.05 < P \leq 0.10$.

Results

Effect of breed on serum binding *Hc* eggs and subsequent hatch rate

Secondary antibodies to sheep IgA (Figure 1A) and IgG (Figure 1B) were used to quantify binding to the egg-stage of *Hc* after serum exposure. IgA derived from Texel and St. Croix sheep had greater binding to eggs than IgA derived from Suffolk sheep ($P < 0.05$, Figure 1C). IgG derived from Texel sheep was reduced compared to the St. Croix and Suffolk treatments ($P < 0.05$, Figure 1D). However, the magnitude of overall differences was less for IgG compared to IgA binding.

Egg hatch was reduced in Texel sheep *in vitro* (Figure 1E). Given observed breed effect of IgA and IgG binding to *Hc* eggs, subsequent egg hatch was investigated when exposed to serum and peripheral blood mononuclear cells (PBMC). Serum and PBMC from the three respective breeds were added to eggs isolated from Suffolk lambs. There was no effect of serum ($P = 0.87$). However, those eggs exposed to Texel PBMC had reduced hatch rates compared to both the St. Croix and Suffolk treatments ($P < 0.01$). Even so, the change in hatch rate does not explain the FEC reduction observed in Texel sheep. Therefore, immune response to adult-stage *Hc* was investigated.

In vivo comparison of *Hc* infectivity in Suffolk and Texel lambs

Characteristics of *Hc* infection differ in Texel sheep compared to parasite-susceptible Suffolk sheep. Five Suffolk lambs and five Texel lambs were infected with 10,000 *Hc* L3. Fecal egg count segregated by breed starting at week three and continued to harvest (Figure 2A). Packed cell volume did not differ over the experiment (Figure 2B). At harvest, Texel sheep had numerically half the number of adult worms as Suffolk sheep but this difference was not

significant ($P = 0.15$, Figure 2C). Even so, an adult worm infection was established in all Suffolk and Texel lambs with the majority of lambs at over 500 adult worms (Figure 2D). Additionally, abomasal lymph nodes were enlarged in Suffolk lambs ($P < 0.05$, Figure 2E) with Suffolk and Texel lambs having a similar number of lymph nodes (Figure 2F).

Adult worms were separated by sex and worm length was measured. Male worms were shorter than females as expected ($P < 0.01$, Figure 2G). Yet, in both sexes, worms harvested from Texel sheep were shorter than worms harvested from Suffolk sheep ($P < 0.01$). When FEC was regressed on adult worm burden, greater oviposit per adult worm was seen in the Suffolk sheep but this was not significant ($P = 0.20$, Figure 2H). Approximately 2.9 eggs/g could be attributed to each adult in Suffolk lambs compared to 1.6 eggs/g per adult worm in Texel lambs. This difference represents infection status of 5 lambs per breed. Greater breed representation may have increased the power of this test.

Adult Worm Cellular Binding

Cellular binding to the head and reproductive structures of both male and female worms were enhanced in Texel and parasite-resistant St. Croix sheep. Adult worms were harvested from parasite-susceptible Suffolk sheep and incubated with PMBC and serum from the three respective breeds *in vitro*. Greater binding around the head of adult *Hc* was observed with Texel (Figure 3C) and St. Croix (Figure 3A) treatments. No binding was observed in the worms exposed to Suffolk cells and serum (Figure 3B).

The genital pore region of the adult female is used for both copulation and oviposit. Here, greater binding was present around the flap and pore region in the Texel (Figure 3F) and St.

Croix (Figure 3D) sheep. Very little binding was observed around the genital pore in the Suffolk lambs (Figure 3E).

The bursa in male worms is used for copulation. The bursa in worms exposed to Texel (Figure 3I) and St. Croix (Figure 3G) cells and serum were closed and restricted with some binding around the exterior. In Suffolk treatments, the bursa was open and appeared functional (Figure 3H).

To validate *in vivo* findings of reduced worm fecundity and explain a possible mechanism associated with cellular-mediated obstruction of egg release, oviposit from adult worms was evaluated *in vitro*. Adult *Hc* harvested from a Suffolk lamb were incubated with PBMC and serum from St. Croix, Suffolk, and Texel sheep for 24 hours and egg release quantified. Egg release tended to be reduced in St. Croix and Texel treatments ($P = 0.09$, Figure 3J) supporting *in vivo* findings. Further, intrauterine egg hatch was observed in adult worms exposed to Texel PBMC and serum. Normally, adult female *Hc* release eggs which are deposited into the environment in fecal matter. Egg hatch and larval development ensues exterior to the host. Intrauterine egg hatch in live females suggests obstruction of egg release by Texel PBMC and serum further supporting evidence of reduced worm fecundity.

Discussion

Data presented here elucidate a mechanism for documented parasite resistance in Texel sheep. Previous work with parasite resistance in Texel sheep has described genomic markers such as the MHCII haplotype DRB1*1101 associated with decreased IgA activity, L4 numbers and FEC (Ali et al., 2019). However, these associations do not provide a specific mechanism connecting the marker and observed phenotype.

In the standard model of parasite resistance, St. Croix sheep produce a more rapid cellular and humoral response after infection, which indicates an improved ability to recognize and respond to the L3 stage of the parasite (Bowdridge et al., 2013; Bowdridge et al., 2015). Early IL-4 production was increased in the St. Croix, indicating Th2 polarization (Jacobs et al., 2015; Jacobs et al., 2018). This Th2 polarization is associated with increased smooth muscle contractility and larval expulsion (Zhao et al., 2008; Horsnell et al., 2011). Additionally, neutrophil and macrophage responses to infection result in extracellular trap formation, decreased larval ATP and motility (Shepherd et al., 2017; Garza et al., 2018). Taken together, St. Croix are able to recognize and respond to L3 of *Hc* producing an unfavorable environment for establishment in the abomasum.

In the Texel, however, adult worm establishment is permitted, but reduction in FEC creates a disparity with traditional models of parasite resistance. Here, a cellular and humoral mechanism rooted in the control of worm fecundity may explain this disparity. Previously, worm size has been positively associated with worm fecundity (Lacroux et al., 2006; Rowe et al., 2008). Worm length is greater in B cell deficient mice infected with *Heligmosomoides polygyrus*. This variation in worm size and consequently in worm fecundity may be regulated by host immune response (Stear et al., 1995; Stear et al., 1999; Liu et al., 2010). Increased cellular binding in the head region of the adult worm may compromise feeding ability. Consequently, binding such as that observed with Texel PBMC and serum could be a limiting factor in worm size. Impact of worm size may not be the only limiting factor on worm fecundity; binding the reproductive regions may impede egg release. In adult females exposed to Texel PBMC and serum, intrauterine egg hatch was observed.

Intrauterine egg hatch, also known as matricidal hatching or worm bagging, has been described in the free-living nematode *Caenorhabditis elegans* (Trent et al., 1983). In *C. elegans,* this mechanism is used in times of stress such as starvation, a change in chemical environment or bacterial exposure to improve larval survival (Edward Caswell-Chen and Jianjun Chen, 2003; Calafato et al., 2008; Mosser et al., 2011). Adult females sacrifice their body nutrients to feed the larvae and improve the number which reach the hardier dauer stage. This mechanism is known as facultative vivipary (Chen and Caswell-Chen, 2004). Matricidal hatching also has been reported in the plant dwelling nematodes *Bursaphelenchus xylophilus* and *Heterorhabditis bacteriophora* (Abelleira et al., 2017; Santhi et al., 2017).

However, no previous reports of matricidal hatching of helminth parasites in ruminant livestock were found. In *C. elegans,* this mechanism has genetic controls and is utilized as an evolutionary survival mechanism in which the adult female sacrifices herself to supply nutrients for larval development (Waggoner et al., 1998). In *Hc,* matricidal hatching may be occurring due to cellular binding impeding ovipositing while the adult female is still alive, rather than an evolutionally mechanism for species survival. This may be the consequence of a host defense system for control of worm fecundity.

IgA binding to the egg-stage of *Hc* was greater in St. Croix and Texel sheep yet egg hatch was independent of serum status. Lambert et al. (2015) also observed little association between egg-specific antibody and hatch rates of *Trichostrongylus retortaeformis* and *Graphidium strigosum* in rabbits. Introduction of Texel PBMC reduced hatch rate of *Hc* eggs. Even so, helminth eggs hatch external to the host, so PBMC impact on egg hatch rate would be independent of FEC.

A proposed mechanism for the Texel immune response is shown in Figure 4. Briefly, the traditional understanding of parasite resistance or susceptibility is observed between the St. Croix and Suffolk, respectively. The delayed cellular and humoral response to *Hc* in Suffolk sheep results in adult worm establishment. In the St. Croix, early response to larval stages prevents adult worm establishment and consequent development of a FEC. The Texel sheep allows adult worm establishment as shown by Figure 3D. Yet, FEC is reduced compared to the Suffolk. Thus, the Texel immune response is oriented towards the adult-stage, reducing worm fecundity. Taken together, reduced FEC in Texel sheep may reduce pasture contamination and reinfection, limiting parasite burden over time. Additionally, improved market acceptability of Texel sheep compared to traditional parasite-resistant breeds make the Texel a viable terminal sire option for forage-based production systems.

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Tables and Figures

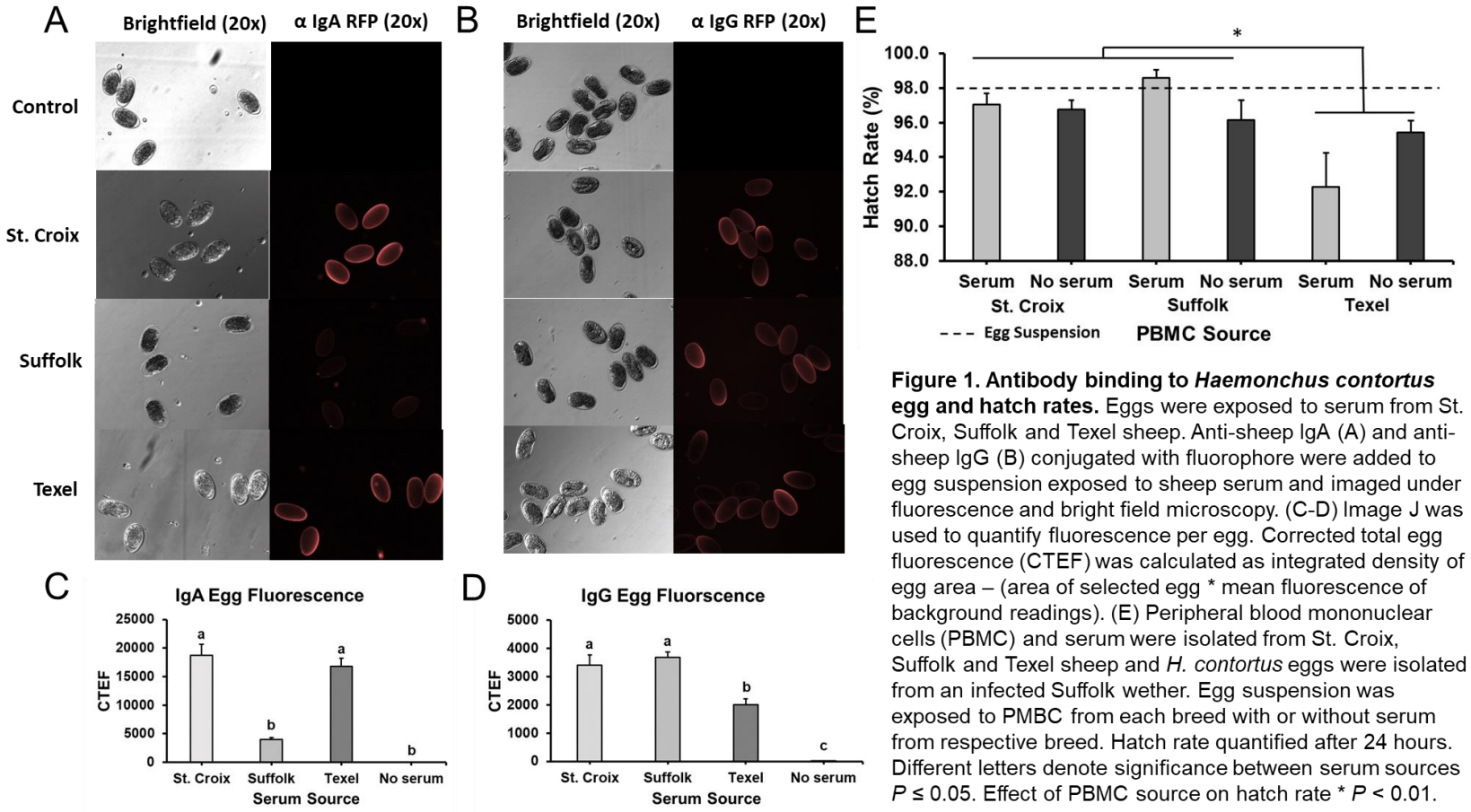

Figure 1. Antibody binding to *Haemonchus contortus* egg and hatch rates. Eggs were exposed to serum from St. Croix, Suffolk and Texel sheep. Anti-sheep IgA (A) and anti-sheep IgG (B) conjugated with fluorophore were added to egg suspension exposed to sheep serum and imaged under fluorescence and bright field microscopy. (C-D) Image J was used to quantify fluorescence per egg. Corrected total egg fluorescence (CTEF) was calculated as integrated density of egg area – (area of selected egg * mean fluorescence of background readings). (E) Peripheral blood mononuclear cells (PBMC) and serum were isolated from St. Croix, Suffolk and Texel sheep and *H. contortus* eggs were isolated from an infected Suffolk wether. Egg suspension was exposed to PMBC from each breed with or without serum from respective breed. Hatch rate quantified after 24 hours. Different letters denote significance between serum sources $P \leq 0.05$. Effect of PBMC source on hatch rate * $P < 0.01$.

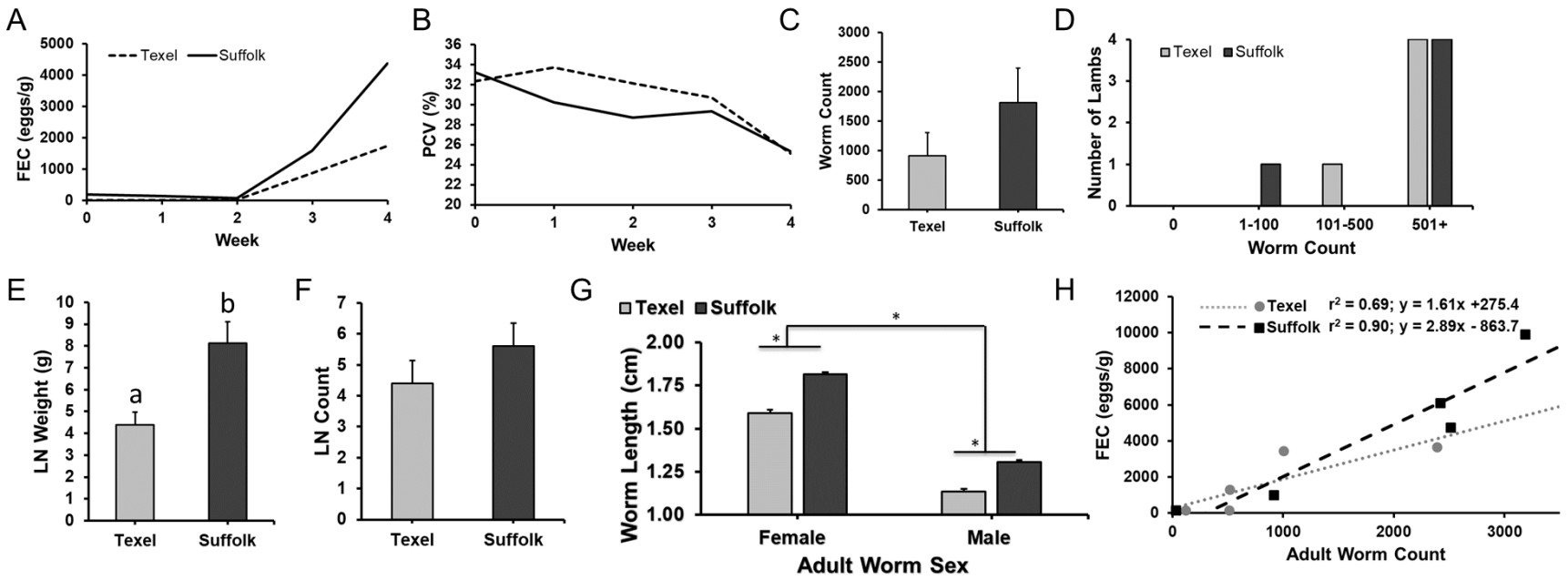

Figure 2. *Haemonchus contortus* infection in Texel and Suffolk sheep. (A) Texel and Suffolk lambs (n = 5/breed) were infected with 10,000 L3 *H. contortus* larvae. The infection was allowed to establish for 30 days. Fecal egg count (A, FEC) and packed cell volume (B, PCV) was measured every 7 days. (C) Lambs were harvested on day 30 after infection. Adult worms were collected. Total worm counts were determined in each lamb. (D) Distribution of worm counts by breed. (E-F) Lymph nodes were collected from the lesser curvature of the abomasum and weighed. (G) Adults worms (n = 100/lamb) were randomly sampled from each lamb. Worms were sorted by male and female and measurements of worm length were made using Image J. (H) Fecal egg count (FEC) and adult worm count were measured at harvest. A regression was utilized to determine the number of eggs/g which can be attributed to each adult worm. Different letters indicate significance $P \leq 0.05$. Effect of breed and worm sex on worm length * $P < 0.05$.

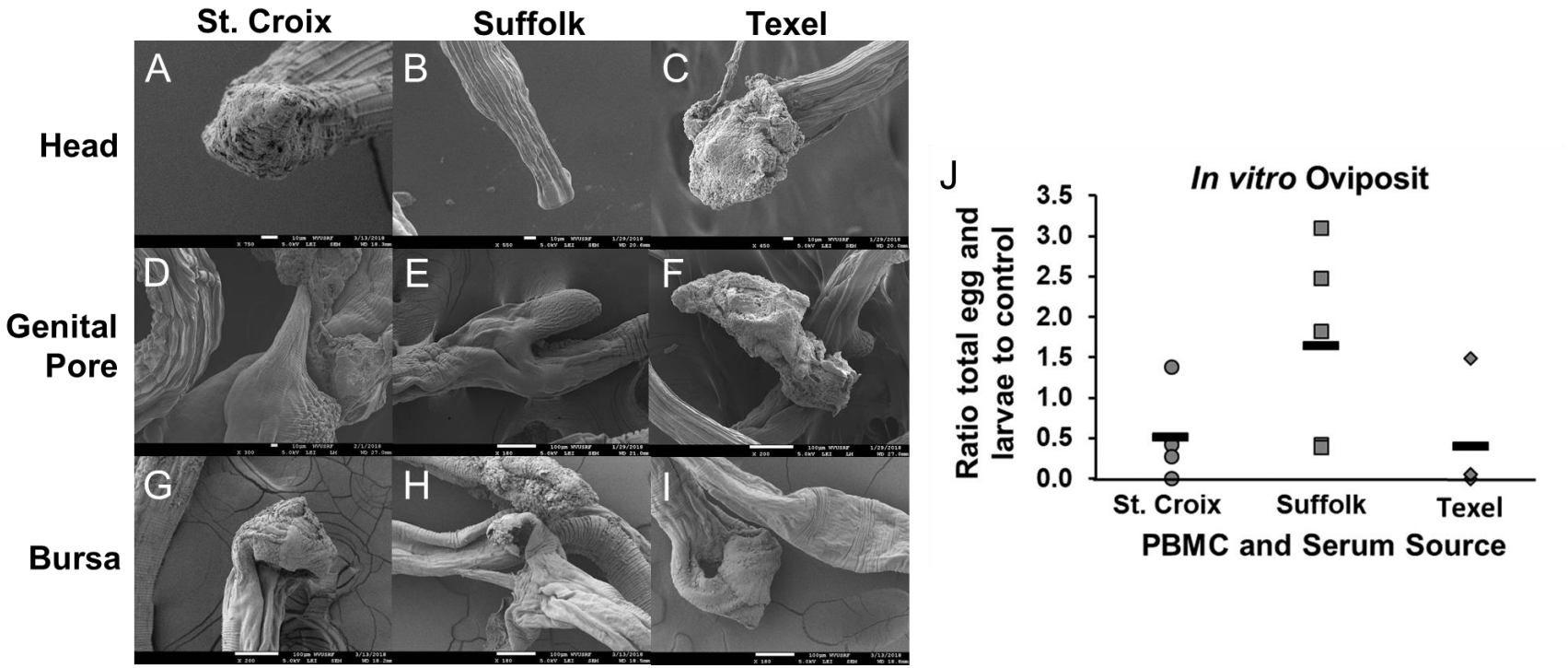

Figure 3. Cellular binding to adult *Haemonchus contortus in vitro* and subsequent egg release. Peripheral blood mononuclear cells (PBMC) and serum were isolated from St. Croix, Suffolk, and Texel sheep. Adult worms were harvested from a Suffolk wether. Adult female and male worms were incubated for 24 hr with PBMC and serum from respective breeds. Worms were fixed and scanning electron microscopy was used to image head (A-C), genital pore of females (D-F) and bursa of males (G-I). (J) Adult-stage *H. contortus* were incubated with peripheral blood mononuclear cells (PBMC) and serum from St. Croix, Suffolk and Texel sheep. Egg release was quantified as total eggs and hatched larvae per female worm after 24 hr. Counts were standardized to control wells. Solid lines indicate averages for each breed. Breed effect $P = 0.09$.

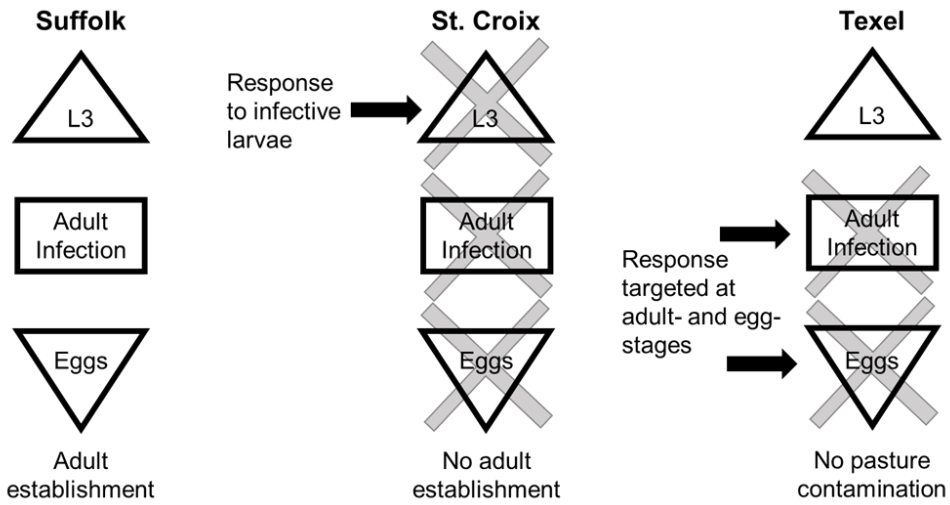

Figure 4. Proposed mechanisms for *Haemonchus contortus* resistance in St. Croix and Texel sheep compared to absence of resistance in Suffolk sheep. St. Croix response based on data from Bowdridge et al., 2013; 2014; Jacobs et al., 2015. Texel sheep allow larval establishment (Figure 3). Immune response is oriented to the adult- and egg-stages. Worm fecundity and FEC is reduced.

CHAPTER III: EFFECT OF SIRE FECAL EGG COUNT ESTIMATED BREEDING VALUE ON KATAHDIN LAMB PASTURE PERFORMANCE

Abstract

With significant genetic variability exiting within breed for gastrointestinal nematode (GIN) resistance, selection may be an important tool to combat anthelmintic resistance in GIN populations. To better understand selection for parasite resistance based on the fecal egg count (FEC) estimated breeding value (EBV), a divergent mating scheme was established. Over two years, Katahdin rams with exceptionally high (High FEC; n = 5) or low (Low FEC, n = 5) FEC EBV were mated to random groups of Katahdin ewes at the Southwest Virginia Agricultural Research and Extension Center (Glade Spring, VA). Lambs were born mid-March and managed as one contemporary group (Weaning: June 4). In Year 1 (YR1), FEC was collected on all lambs June 26 with no prior anthelmintic treatment. In Year 2 (YR2), beginning at 60 days of age, body weights and FAMACHA scores were collected weekly and FEC biweekly. Anthelmintic administration occurred based on FAMACHA ≥ 3 in YR2. Lamb survival determination excluded first 7 days of age. Statistical analysis was performed using SAS (SAS Institute, Cary, NC) with fixed effects of sire type. Lamb FEC EBV corresponded to sire type validating the mating scheme. Lamb FEC was similar and variable prior to and shortly after weaning. After this point, High FEC-sired lambs had greater FEC compared to Low FEC-sired lambs ($P < 0.05$) and anthelmintic treatment corresponded to FEC ($P < 0.05$). In YR1, death losses were greater for High FEC-sired lambs ($P < 0.05$) and those lambs that died had greater FEC EBV than those that survived ($P < 0.05$). In YR2, post-weaning FEC EBV difference between High FEC-sired lambs that survived to 120 days of age and those that died was significant (73% vs. 138%, $P < 0.01$).

Therefore, magnitude of FEC EBV may be used as a predictor of immunological fitness beyond resistance to GIN infection.

Introduction

Gastrointestinal nematodes (GIN) are a major challenge to sheep production globally. These GIN infections result in reduced performance and significant economic losses (Sackett et al., 2006; Mavrot et al., 2015). Further production challenges result from anthelmintic resistance in worm populations (Howell et al., 2009). Therefore, an integrated approach to parasite management may be a more sustainable solution. The combination of nutritional management (Mata-Padrino et al., 2019), selective deworming (Kaplan et al., 2004), anthelmintic alternatives (Burke and Miller, 2006; Burke et al., 2016) and genetic selection could help mitigate anthelminthic needs.

There is significant variability in genetic resistance to internal parasitism. This variability exists within and among breeds (Notter et al., 2003; Vanimisetti et al., 2004b; Notter et al., 2007). Fecal egg count (FEC) is a reliable indicator of parasitism with moderate heritability (Vanimisetti et al., 2004a; Ngere et al., 2018). Estimated breeding values (EBV) for FEC have been available through the National Sheep Improvement Program (NSIP) since 2003 (Notter et al., 2007). The FEC EBV is expressed as a percentage change in FEC. Negative FEC EBV indicates the genetic merit to reduce FEC (Notter and Lewis, 2018). Selection for reduced FEC in Australian Merinos has resulted in improved parasite resistance in progeny and lower periparturient rise (PPR) in ewes (Woolaston et al., 1990; Woolaston, 1992). This is an example of successful selection for disease resistance.

Selection for disease resistance can be challenging due to the cost associated with identification and measurement of indicator traits (Snowder, 2006). If genes controlling disease resistance are not mutually exclusive, then selection for resistance to one disease could improve resistance to other diseases, resulting in improved general immunity. In a quantitative form, this is expressed as co-heritability ($r_g h_1 h_2$) where r_g is the genetic correlation between traits and h_1 and h_2 are the square roots of the heritability for disease traits. When comparing economically relevant diseases in the Merino, positive coheritability existed for parasite resistance compared to foot rot and dermatophilosis (Raadsma et al., 1997). However, beyond this Merino flock, little evidence exits of general immunity and correlative responses to selection for disease resistance.

The Katahdin hair sheep was developed by Michael Piel of Maine during the late 1950's from crosses of "African Hair Sheep" and traditional wool breeds (Breed Origin & History, 2019). These "African Hair Sheep" were imported from the Caribbean island of St. Croix and were likely Virgin Island Whites, a predecessor of the St. Croix breed (Wildeus, 1997). The Katahdin has since become the most registered breed in the U.S. (Morgan, 2019). Known for its hair phenotype and forage-adaptability, the breed has traditionally been considered parasite resistant. However, significant variability exists based on the FEC EBV (NSIP Searchable Database, 2019). Thus, challenges arise in selecting for resistance and management of lambs in forage-based production systems with significant parasite exposure.

Lambs are most susceptible to parasitism during their first exposure. Primary GIN infections are more severe than subsequent challenge infections (Jacobs et al., 2015). In a forage-based system, this exposure occurs when lambs begin grazing. In spring lambing flocks, weaning and associated lamb stress coincides with GIN establishment and the pathologies resulting from

the primary GIN infection. Therefore, improved selection of lambs in forage-based production systems to reduce parasitism around the time of weaning could be beneficial.

The aim of this study was to assess lamb fitness around the time of weaning in the context of parasitism and survivability. The relationship between parasitism and susceptibility to other pathogens will be examined using lambs bred to be parasite susceptible or parasite resistant based on FEC EBV. It is hypothesized that young lambs bred to extreme positive or extreme negative FEC EBV rams will have corresponding FEC. Additionally, disease fitness is expected to be correlated negatively with individual FEC EBV.

Materials and Methods

Breeding Scheme

Rams were sourced from industry flocks participating in NSIP. Registered Katahdin sires were selected based on FEC EBV. With the exception of one sire (High Sire 5 in Year 2 (YR2)), only proven rams were utilized and EBV accuracy for FEC traits was over 0.70. Index values (USA Hair; Notter and Lewis, 2018) were used to ensure sires were relatively similar in genetic merit beyond FEC. In general, index values varied from 103.9 to 106.3. High Sire 5 had an index score of 112.5 but was needed for equal representation of sire EBV types. In Year 1 (YR1), a total of 4 sires were used with exceptionally high (High FEC) or exceptionally low (Low FEC) FEC EBV (Table 1). In YR2, 8 sires were used. High Sire 1 and Low Sire 1 from YR1 were utilized again in YR2 for connectedness. Average difference in post-weaning FEC (PFEC) EBV between sire groups in YR1 was 300.9% and in YR2, PFEC difference was 421.0%.

Rams were mated to the ewe flock at the Southwest Virginia Agricultural Research and Extension Center (SWAREC, Glade Spring, VA). Registered Katahdin ewes (YR1, n = 119;

YR2, n = 137) were randomly assigned to service sire mating groups with even distribution of ewe age (Table 1). Ewe age ranged from lamb (approximately 7 months) to 9 years of age. Half-sibling matings were avoided. Ewe PFEC EBV was similar between Low FEC and High FEC mating groups (YR1: -34 vs. -39%; YR2: -21 vs. -34%; respectively). Inbreeding in resulting progeny was negligible (< 3%). Ewes were exposed to rams for natural mating beginning October 15 in each year. Sires were removed from breeding groups after approximately 45 days. In YR1, 97 ewes lambed. In YR2, 104 ewes lambed (Table 2).

Management

Timeline for lamb management and data collection is outlined in Figure 1. Lambs were born at the SWAREC from March 9 to April 29 in YR1 with an average lambing date of March 19 (Table 2). In YR2, lambing date ranged from March 13 to April 13 with an average lambing date of March 24 (Table 2). Lambs were jugged at birth and returned to pasture with their dam after 24-48 hours. Lambs were managed on fescue-based mix forage pasture until weaning at approximately 70 days of age. The flock was managed as one contemporary group and rotated among paddocks based on forage availability. Lamb deaths were recorded, and cause of death identified if possible. At weaning (June 4 in each year), lambs were moved to a clean (ungrazed) pasture and managed for an additional 30-45 days. In YR1, lambs were vaccinated for *C. perfringens* types C and D as well as *C. tetani* (Bar Vac® CD/T) at approximately 45 days of age and a booster given the day of weaning. In YR2, lambs were vaccinated at approximately 60 days of age and the booster was given one week after weaning. Additionally, lambs were vaccinated for *C. perfringens* type A (Elanco) in YR2. After weaning, lambs were supplemented 2% body weight with a concentrate pellet (13% CP, 75% TDN).

Data Collection

In YR1, lambs were removed from pasture 22 days after weaning (June 26). A fecal sample was collected rectally, and all lambs were dewormed with levamisole hydrochloride (8 mg/kg, Agrilabs, Columbia, MO, USA). Body weights were taken at weaning and used to calculate adjusted weaning weights. Weaning weights were adjusted for lamb and ewe age, sex, and birth/rear type based on Katahdin adjustment factors. June 26 FEC was considered the weaning FEC (WFEC).

To better understand lamb parasitism and death losses around the time of weaning, in YR2, sample collection began at the time of first vaccination (45-60 days of age). Body weights, blood samples and FAMACHA scores were collected weekly until July 9 (approximately 110 days of age). Fecal samples were collected biweekly. At this point, lambs were removed from pasture and treated with levamisole hydrochloride (8 mg/kg, Agrilabs, Columbia, MO, USA) . May 28 FEC was considered WFEC. June 4 weaning weight was used for adjusted weaning weight calculation. Foot scald was assessed weekly by visual evaluation of lameness and treated with gamithromycin (6 mg/kg, Merial) as necessary. Lambs were given anthelmintic treatment at FAMACHA™ score ≥ 3. Levamisole hydrochloride was the primary anthelmintic used. After a lamb was given anthelmintic treatment, subsequent FEC data were removed from analysis.

A modified McMaster test (Whitlock, 1948) was used to measure FEC. Raw egg counts were multiplied by 50 to determine FEC in eggs/g. Lamb death was recorded from birth to removal from pasture for survival evaluation. For analysis, only lamb death after 7 days of age was considered.

Statistical Analysis

Data were analyzed using SAS (SAS Institute, Cary, NC). Following anthelmintic treatment, FEC and PCV data were removed from further analysis to prevent bias in sire types (High FEC, Low FEC) treated more frequently. A log transformation was used on FEC data [ln(FEC+1)] for normality. The Mixed Model procedure of SAS with fixed effects of sire type and random effects of individual sire were used for body weight, FAMACHA score, FEC and EBV data. In YR2, time and sire type X time interaction were included in a repeated measures model for FEC data. Lamb birth and weaning data (body weight and FEC) were processed by Lambplan (Sheep Genetics, Australia) for EBV generation. Lamb FEC EBV was compared by lamb survival status within sire type. Bonferroni's adjustment was used for separation of least-squares means. Significance was determined at $P \leq 0.05$. Tendencies were determined at $0.05 < P \leq 0.10$.

The Genmod procedure was used for deworming frequency, foot treatment and lamb death data with fixed effects of sire type. Deworming frequency and foot treatment data were analyzed as the cumulative total of lambs treated by sire type over the sampling period.

Results

Weaning Performance

Average lambing date and litter size were similar between sire types (Table 2). In YR2, lambs sired by High Sire 4 were approximately two weeks younger than the rest of the lamb crop. At weaning, differences in adjusted weaning weights were similar to those expected based on WWT EBV (Table 3). In YR1, weaning occurred on June 4 and lambs were backgrounded for 3 weeks prior to removal from pasture. On June 26, FEC were collected and all lambs were

dewormed. In YR2, weaning occurred on the same day and lambs were backgrounded until July 9. Anthelmintic treatment began May 14 based on weekly FAMACHA™ score. After anthelmintic treatment, subsequent FEC data were removed from analysis. Lamb FEC EBV validated the selection scheme (Table 3). For phenotypic comparison of FEC between years, in YR2, June 25 FEC was used. Trends in weaning FEC followed those expected by WFEC EBV (Table 3), although the magnitude of FEC differences was less than that predicted by EBV.

To better understand parasitism and death losses around the time of weaning, in YR2, FAMACHA scores were collected weekly and FEC biweekly. Deworming occurred based on FAMACHA score ≥ 3 and is presented as the cumulative percentage of lambs treated with anthelmintic over the period from May 14 to July 9. Prior to weaning, FEC was similar between sire types. After weaning, FEC was variable for approximately 3 weeks and FEC differences between sire types did not follow expected trends based on FEC EBV. After this period, FEC segregated as expected with higher FEC in lambs sired by High FEC sires compared to lambs sired by Low FEC sires ($P < 0.05$, Figure 2A). Comparing sire types, FEC in lambs sired by Low FEC sires (Figure 2B) peaked shortly after weaning and then generally declined until the end of the sampling period. In lambs sired by High FEC sires, FEC remained constant or continued to rise after weaning (Figure 2C).

Anthelmintic treatment frequency was similar between sire types prior to weaning. After weaning, anthelmintic treatment differences segregated. A greater percentage of lambs sired by High FEC sires required treatment after weaning and differences remained consistent through the end of the sampling period ($P < 0.05$, Figure 2D). At the end of the sampling period, the cumulative proportion of lambs treated with anthelmintic was 15% greater in High FEC-sired lambs compared to Low FEC-sired lambs (Figure 3D). Individual sire differences were variable

and ranged from 39% of lambs sired by Low Sire 3 to 89% of lambs sired by High Sire 3 (Figure 3G).

Survivability

Significant death losses occurred in YR1 with few clinical symptoms prior to death. Veterinary diagnosis suggested *C. perfringens* type A. Lambs sired by High FEC sires had greater death losses than lambs sired by Low FEC sires (29.9% vs 10.6%, respectively; $P < 0.05$, Figure 3A). Due to a general tendency of lamb loss prior to 7 days of age, unrelated to sire selection, these data were excluded from survivability analysis. In YR1, FEC data were not collected until late June so it is unclear if lamb losses in spring and early summer were associated with parasitism. However, FAMACHA™ scoring was used by SWAREC staff to monitor anemia levels (unreported) and no concern for parasitism appeared during this time period.

Lamb loss was lower in High FEC-sired lambs in YR2 compared to YR1. However, lamb loss was similar in Low FEC-sired lambs in both years (Figure 3A). In YR2, lambs were vaccinated for *C. perfringens* type A in addition to *C. perfringens* C and D and *C. tetani*. Vaccination appeared to reduce lamb loss in High FEC-sired lambs. Lamb loss in Low FEC-sired lambs may represent standard losses expected in this management system and environment.

In both years, treatment for foot scald was determined based on lameness assessed weekly. Foot treatments were variable between years and sires and was not consistent with sire type (Figure 3B-C). In YR1, foot treatments ranged from 16% in High Sire 2 to 44% in Low Sire 2 (Figure 3E). In YR2, foot treatments ranged from 38% in Low Sire 5 to 83% in High Sire 3

(Figure 3F). Sires with the lowest percentage of lambs treated in both years were raised in close geographic proximity to the research station.

EBV and Survivability Comparisons

Lamb EBV were generated based on pedigrees and lamb performance through this weaning period. Fecal egg count EBV was used to compare selection for parasite resistance and survivability. Weaning FEC and PFEC EBV averages between those of lambs that died in each year and those that survived are shown in Figure 4A and 4D. Lambs that did not survive had greater WFEC and PFEC EBV in YR1 ($P < 0.05$).

Further, survival differences and FEC EBV were compared by sire type (Figure 4B and 4C, 4E and 4F). In general, the magnitude of FEC EBV difference between the lambs which survived and those lambs which died was greater for High FEC-sired lambs than Low FEC-sired lambs. In YR2, Low FEC-sired lambs that died had a WFEC EBV of -48.3% compared to -41.9% for those that survived, a difference of 6.4% (Figure 4B). In contrast, for High FEC-sired lambs, those lambs that died had a greater WFEC EBV than those that survived (83.1% vs. 36.8%, $P < 0.05$; Figure 4C). A similar trend existed for PFEC EBV (Figure 4F) as High FEC-sired lambs that died had a greater PFEC EBV compared to those that survived (137.6% vs. 73.2%, $P < 0.05$). The difference within Low FEC-sired lambs was negligible. Taken together, lamb loss when using Low FEC EBV rams is unpredictable. Losses are environment-dependent and occur regardless of lamb FEC EBV. However, when using High FEC EBV rams, higher death losses may be realized in those lambs with more extreme positive FEC EBV.

Discussion

Parasitism is a significant hindrance to lamb production in forage-based production systems. Lambs are generally more susceptible to infection than mature sheep (Notter et al., 2017). This, in combination with stress associated with weaning, can result in a rise in GIN infection and consequent pathologies such as anemia, hypoproteinemia, and death in severe infections (Zajac, 2006). Additionally, disease challenge in the grazing environment can result in further losses.

Clostridium perfringens is associated with enterotoxemia or overeating disease (Simpson et al., 2018). *C. perfringens* type C and D are most common; however, increased incidence of type A has been observed in regional proximity to the SWAREC. Prior to vaccination for type A (YR1), significant lamb loss was observed from 7 days of age until approximately 100 days of age in High FEC-sired lambs. After vaccination, death losses between High and Low FEC-sired lambs were more similar. However, in both years, losses from lambs sired by Low FEC rams were consistently around 10%. Given selection for reduced FEC and improved parasite resistance, death losses of around 10% could be expected for this management system and environment. Death losses above that observed in Low FEC-sired lambs could be a result of compromised immunity and increased susceptibility to parasitism and possibly other pathogens. In Soay sheep populations, increased parasite-specific antigen concentration was associated with improved overwinter survivability (Watson et al., 2016). The ability to develop an adaptive response to the GIN may be an indicator of host fitness.

The improved survival of High FEC-sired lambs after vaccination for *C. perfringens* type A in YR2 supports *C. perfringens* type A as the causative agent resulting in the death losses observed in YR1. These data indicate that High FEC-sired lambs were unable to respond to the

pathogen in the absence of vaccination. Regardless, death losses were greater than Low FEC-sired lambs in YR2. Additionally, lambs that died had greater FEC EBV; thus within sire type, greater death losses may be observed in High FEC-sired lambs with more extreme FEC EBV. Raadsma et al. (1997) suggested that resistance to parasitism may be weakly correlated with foot rot and dermatophilosis resistance. In Figure 3, incidence of foot scald was variable between years and sires. Thus, consistent relationship with sire type and FEC EBV was lacking.

The divergent mating scheme validated EBV use as a selection tool and predictor of lamb performance. Differences in adjusted weaning weight between sires were small yet comparable to that predicted by WWT EBV. Trends in FEC differences were as predicted by WFEC and PFEC EBV. Removal of FEC data after anthelmintic treatment may have reduced average FEC of High FEC-sired lambs resulting in a lower magnitude of FEC difference between High and Low FEC-sired lambs compared to EBV estimates. Selection for reduced FEC has been successful in Merino populations (Woolaston et al., 1990). Additionally, this selection resulted in a reduction in PPR indicating that similar genes control both lamb parasitism and ewe PPR (Woolaston, 1992; Notter et al., 2018). Genetic relationships between FEC and body weight appear to be small and relatively unrelated (Ngere et al., 2018). Therefore, simultaneous selection for growth and parasite resistance should not be antagonistic.

Fecal egg count variation around the time of weaning should be considered when collecting samples for genetic analysis. The NSIP database reports WFEC (45-90 days of age) and PFEC (91-150 days of age). The variation in FEC and deviation from expected trends in the time period up to 3 weeks post-weaning could result in inaccurate WFEC EBV if data are submitted from samples collected in this time period. Rather, WFEC should be collected shortly

before or on the day of weaning. If unable, sample collection should wait until at least 3-4 weeks post-weaning.

In conclusion, the FEC EBV is a reliable predictor of lamb FEC and resistance to parasitism. Data collection for generation of FEC EBV should be restricted from the period beginning at weaning to 3-4 weeks post-weaning to limit FEC inaccuracy from environmental stress. The FEC EBV is also associated with lamb survival. Greater lamb mortality was observed in High FEC-sired lambs and lambs with greater individual FEC EBV. The FEC EBV could be a predictor of lamb survival in forage-based production systems. Possible implications of selection for FEC EBV could be improved lamb fitness and survival in the midst of a variety of pathogenic challenges.

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Tables and Figures

Table 1. Sire summary for Year 1 (YR1) and Year 2 (YR2) matings with estimated breeding values (EBV).

			EBV[3]				
Sire ID[1]	YR1 Ewes[2]	YR2 Ewes[2]	WWT (kg)	PWWT (kg)	WFEC (%)	PFEC (%)	USA Hair
Low Sire 1	30	17	3.6	6.0	-20.6	-67.8	104.5
Low Sire 2	30	-	2.1	3.1	-37.4	-81.5	106.1
Low Sire 3	-	18	2.4	4.6	-90.8	-99.5	106.3
Low Sire 4	-	17	0.1	-1.1	-99.4	-99.1	104.2
Low Sire 5	-	17	1.0	2.0	-11.0	-78.7	103.9
Low FEC Average	**60**	**69**	**1.8**	**2.9**	**-51.8**	**-85.3**	**105.0**
High Sire 1	29	17	0.4	1.3	288.5	348.8	105.1
High Sire 2	30	-	1.8	3.7	47.0	103.5	105.8
High Sire 3	-	17	2.5	3.6	135.9	509.7	105.1
High Sire 4	-	17	1.8	2.4	22.0	120.4	105.1
High Sire 5	-	17	0.9	1.7	205.0	359.8	112.5
High FEC Average	**59**	**68**	**1.5**	**2.5**	**139.7**	**288.4**	**106.7**
Sum/Difference[4]	**119**	**137**	**0.4**	**0.4**	**191.5**	**373.8**	**1.7**

[1]Sire ID denotes grouping based on FEC EBV. Two sires in each group were utilized for breedings in YR1. Three new sires along with a carryover sire were used in YR2.

[2]Represents number of ewes mated to each sire in each year.

[3]Estimated breeding values based on 6/15/2019 Lambplan analysis; weaning weight (WWT), post-weaning weight (PWWT), weaning fecal egg count (WFEC), post-weaning fecal egg count (PFEC), and USA Hair index.

[4]Sum of ewe numbers. Difference in low and high FEC sire EBV.

Table 2. Lambing summary

Sire ID[1]	Ewes[2]	Lambing Date[3]	NLB[4]	NLW[5]
Year 1				
Low Sire 1	22	3/20/2018	1.8	1.7
Low Sire 2	25	3/17/2018	1.8	1.5
Low FEC Average	**47**	**3/18/2018**	**1.8**	**1.6**
High Sire 1	25	3/20/2018	2.3	1.4
High Sire 2	25	3/20/2018	1.8	1.7
High FEC Average	**50**	**3/20/2018**	**2.0**	**1.5**
Sum/Average[6]	**97**	**3/19/2018**	**1.9**	**1.6**
Year 2				
Low Sire 1	13	3/20/2019	2.2	1.8
Low Sire 3	12	3/24/2019	2.1	1.8
Low Sire 4	14	3/23/2019	2.2	1.9
Low Sire 5	11	3/21/2019	2.2	1.8
Low FEC Average	**50**	**3/22/2019**	**2.2**	**1.8**
High Sire 1	15	3/27/2019	1.9	1.7
High Sire 3	14	3/22/2019	1.7	1.4
High Sire 4	12	4/6/2019	1.9	1.8
High Sire 5	13	3/23/2019	2.1	1.6
High FEC Average	**54**	**3/27/2019**	**1.9**	**1.6**
Sum/Average[6]	**104**	**3/24/2019**	**2.0**	**1.7**

[1]Sire ID denotes grouping based on FEC EBV. Two sires in each group were utilized for breedings in YR1.

[2]Represents number of ewes lambing to each sire.

[3]Average lambing date for ewes mated to each sire.

[4]Number of lambs born (NLB). Average number of lambs born per ewe lambing sired by each ram.

[5]Number of lambs weaned (NLW). Average number of lambs weaned per ewe lambing sired by each ram.

[6]Sum of ewe numbers, average lambing date and NLB or NLW.

Table 3. Lamb performance summary with lamb estimated breeding value (EBV)

	Lamb EBV[2]						
Sire ID[1]	WWT (kg)	PWWT (kg)	WFEC (%)	PFEC (%)	USA Hair	Adj. WWT[3] (kg)	FEC[4] (eggs/g)
Year 1							
Low Sire 1	2.3	3.8	-8.0	-40.6	103.6	16.6	2443
Low Sire 2	1.6	2.4	-38.7	-68.0	105.0	16.4	1134
Low FEC Average	**2.0**	**3.1**	**-23.4**	**-54.3**	**104.3**	**16.5**	**1821**
High Sire 1	0.7	1.5	90.8	101.7	104.7	15.1	3029
High Sire 2	1.5	2.9	6.6	21.1	104.5	16.2	2825
High FEC Average	**1.1**	**2.2**	**48.7**	**61.4**	**104.6**	**15.7**	**2914**
Difference[5]	**0.9**	**0.9**	**72.1**	**115.7**	**0.3**	**0.8**	**1093**
Year 2							
Low Sire 1	2.3	3.9	-10.2	-41.6	104.7	18.1	2150
Low Sire 3	1.8	3.3	-66.1	-84.6	105.8	18.6	1815
Low Sire 4	0.6	0.5	-81.1	-80.4	103.5	16.4	1313
Low Sire 5	1.1	2.1	-12.5	-58.3	103.4	16.7	3938
Low FEC Average	**1.5**	**2.5**	**-42.5**	**-66.2**	**104.4**	**17.5**	**2175[a]**
High Sire 1	0.8	1.8	107.7	118.3	104.2	16.1	4064
High Sire 3	1.7	2.6	28.2	114.0	103.9	18.5	1471
High Sire 4	1.6	2.4	-6.6	24.5	105.7	18.3	4517
High Sire 5	0.8	1.5	43.5	71.8	108.3	16.8	2056
High FEC Average	**1.2**	**2.1**	**43.2**	**82.2**	**105.5**	**17.4**	**3398[b]**
Difference[5]	**0.2**	**0.4**	**85.7**	**148.4**	**1.2**	**0.1**	**1223**

Different letters denote significance $P \leq 0.05$ within column.

[1]Sire ID denotes grouping based on FEC EBV. Two sires in each group were utilized for breedings in YR1.

[2]Estimated breeding values based on 6/15/2020 Lambplan analysis; weaning weight (WWT), post-weaning weight (PWWT), weaning fecal egg count (WFEC), post-weaning fecal egg count (PFEC), and USA Hair index.

[3]Weaning weights (WWT) adjusted for lamb and dam age, number of lambs born and weaned and sex.

[4]Year 1 fecal egg count (FEC) based on 6/26/18 FEC. Year 2 FEC based on 5/29/19 FEC.

[5]Difference in low and high FEC sired lambs EBV and performance metrics.

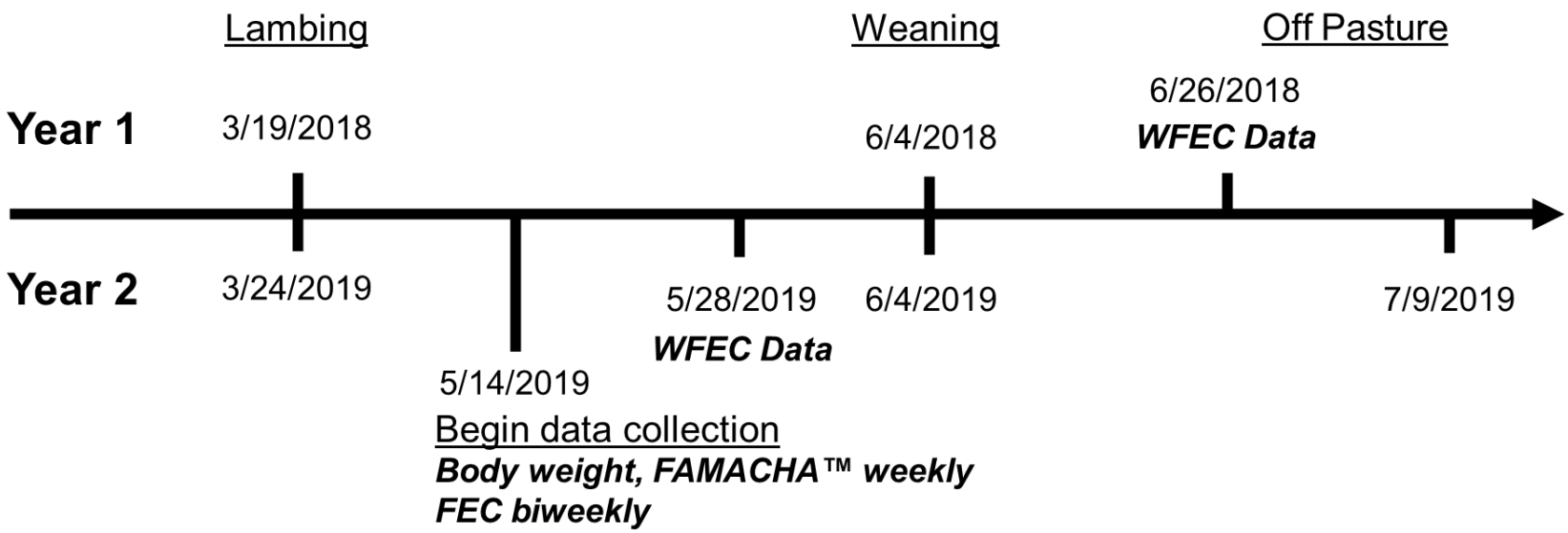

Figure 1. Timeline of lamb management by year. Average lambing, weaning and off pasture dates for each year. In Year 1, weaning fecal egg count (WFEC) data was collected for NSIP data submission on 6/26/2018. In Year 2, body weights, FAMACHA™ scores were collected weekly and FEC biweekly from 5/14/2019 until removal from pasture. WFEC data was collected one week prior to weaning.

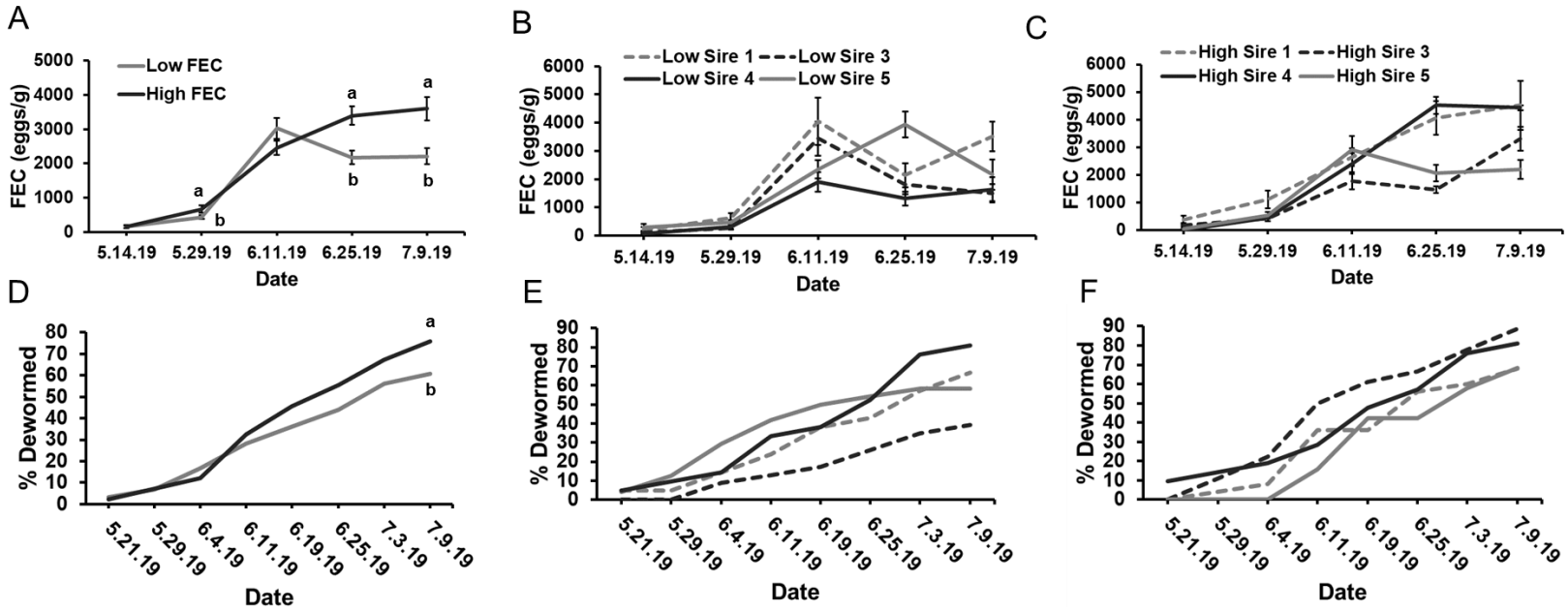

Figure 2. Year 2 fecal egg counts (FEC) and anthelmintic treatment. Lamb FEC by sire type (A) beginning at approximately 60 days of age until removal from pasture. Lamb FEC by Low FEC (B) and High FEC (C) sires. Cumulative deworming by sire type (D) over this period. Once lambs were dewormed, subsequent FEC data were removed from analysis. Deworming data by Low FEC (E) and High FEC (F) sires. Different letters denote significant differences by sire type $P \leq 0.05$.

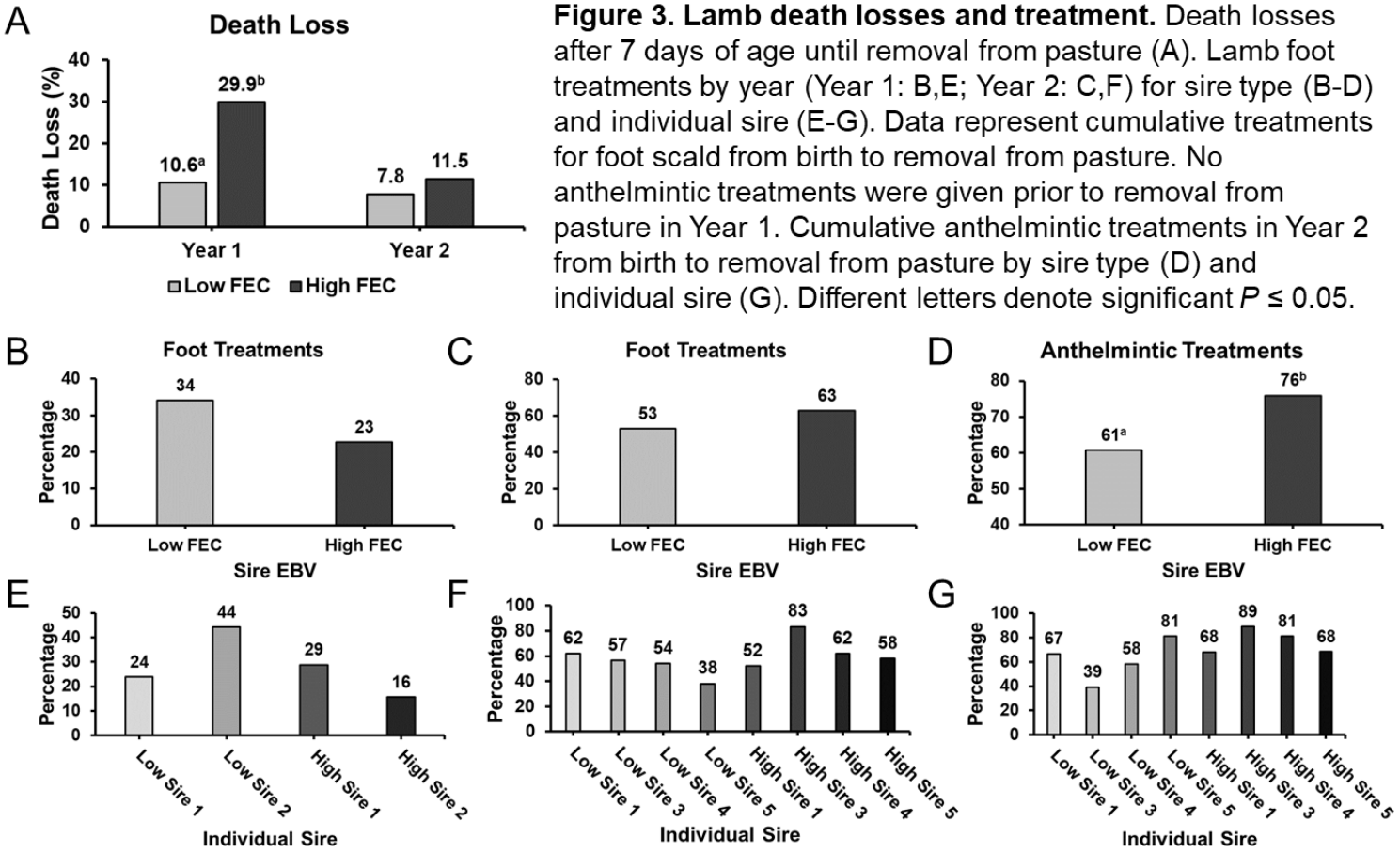

Figure 3. Lamb death losses and treatment. Death losses after 7 days of age until removal from pasture (A). Lamb foot treatments by year (Year 1: B,E; Year 2: C,F) for sire type (B-D) and individual sire (E-G). Data represent cumulative treatments for foot scald from birth to removal from pasture. No anthelmintic treatments were given prior to removal from pasture in Year 1. Cumulative anthelmintic treatments in Year 2 from birth to removal from pasture by sire type (D) and individual sire (G). Different letters denote significant $P \leq 0.05$.

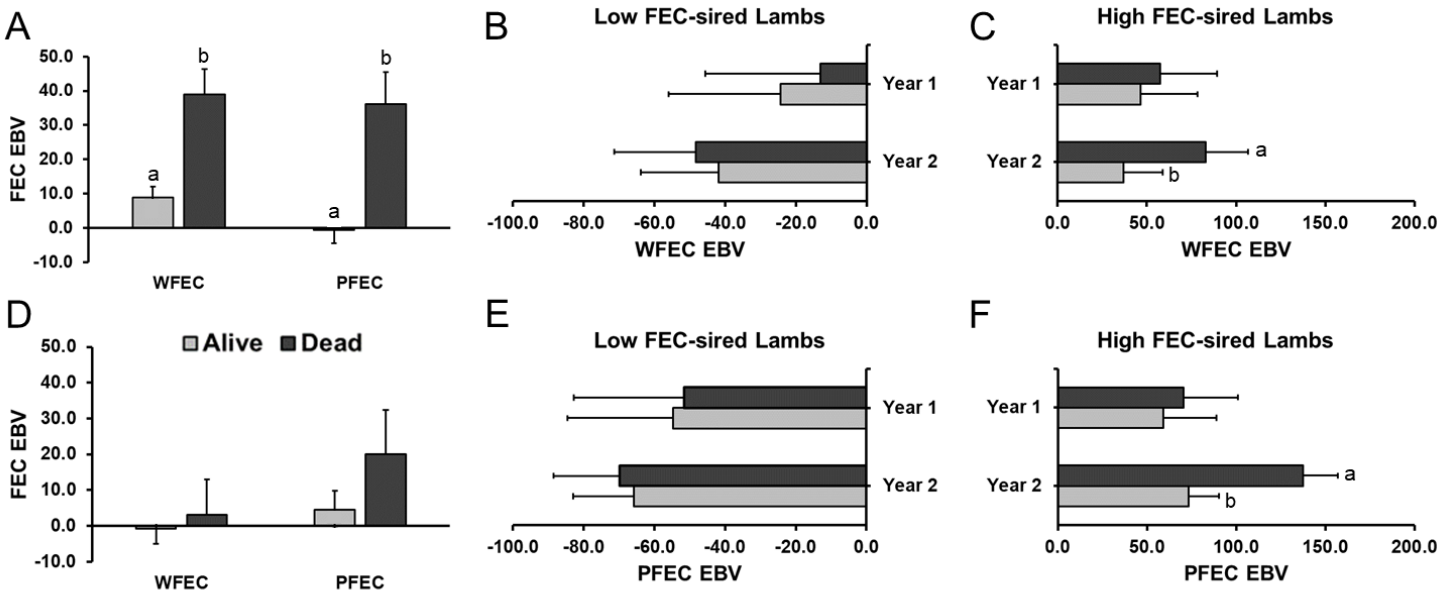

Figure 4. Lamb fecal egg count (FEC) estimated breeding value (EBV) and survivability. Graph A represents Year 1. Graph D represents Year 2. Weaning fecal egg count (WFEC) estimated breeding value (EBV) and post-weaning FEC (PFEC) EBV for live lambs (Alive) and those lambs that died (Dead) prior to removal from pasture (A and D). Lamb WFEC EBV (B and C) and PFEC EBV (E and F) by sire type and year for lambs that lived and those that did not. Different letters denote significance $P \leq 0.05$.

CHAPTER IV: EFFECT OF SIRE FECAL EGG COUNT ESTIMATED BREEDING VALUE ON *HAEMONCHUS CONTORTUS* INFECTION IN KATAHDIN SHEEP

Abstract

In the midst of anthelmintic resistance, genetic resistance to gastrointestinal nematodes (GIN) in lambs could reduce anthelmintic requirements; therefore, the fecal egg count (FEC) estimated breeding value (EBV) was developed as a measure of genetic merit for parasite burden. One of the first breeds to effectively implement the FEC EBV was Katahdin. To better understand the relationship between the FEC EBV and *Haemonchus contortus* (*Hc*) challenge infection, a divergent mating scheme was established with extremely high (High FEC, n = 5) or extremely low (Low FEC, n = 5) FEC EBV Katahdin rams over 2 years. Purebred lambs were born beginning in mid-March and managed on pasture until approximately 120 days of age. A primary infection was established based on FEC during this period. At this point, lambs (n = 118) were removed from pasture, treated with an anthelmintic to reduce FEC and transported to the Animal Sciences Farm at West Virginia University. After a rest period, lambs were given 10,000 (Year 1) or 5,000 (Year 2) *Hc* L3. Body weights, FEC, and packed cell volume were collected weekly. Lambs were harvested at 5 weeks post-infection. Abomasum worm counts were determined and worm length was measured using Image J. Statistical analysis was performed by year using the Mixed Model procedure of SAS (SAS Institute, Cary, NC) with fixed effects of sire EBV type. Change in FEC after the prepatent period was greater in High FEC-sired lambs compared to Low FEC-sired lambs ($P < 0.05$). At harvest, a greater proportion of Low FEC- than High FEC-sired lambs had worm counts of zero ($P < 0.05$). Worm fecundity

was lower in lambs sired by Low FEC rams ($P < 0.05$). Taken together, sire selection for low FEC EBV will lower FEC and worm count and improve GIN resistance in progeny.

Introduction

Parasitism is a significant challenge to sheep production. *Haemonchus contortus* (*Hc*) is a gastrointestinal nematode (GIN) that resides in the abomasum and causes anemia and death in severe infections. Fecal egg counts (FEC) are a quantitative measure of parasitism. Variation in FEC and resistance to GIN infection exists within and among breeds (Notter et al., 2003; Vanimisetti et al., 2004; Notter et al., 2007). Variation in FEC is a phenotypic measurement with genetic underpinnings involving the mechanisms of immunity. A progenitor of Katahdin, St. Croix is well known for immunity. Therefore, instead of selecting for percent St. Croix in Katahdin sheep, selection for low FEC should yield a similar result.

Estimated breeding values (EBV) are measures of genetic merit for various production traits. Breeding values for FEC provide a selection tool for improving parasite resistance by reducing FEC. Heritability for FEC ranges from 0.18 to 0.23 (Ngere et al., 2018). Recording data to calculate FEC EBV has been available to sheep producers since 2003 (Notter et al., 2007). In Katahdin sheep, selection for lower weaning FEC (WFEC) EBV has improved parasite resistance in the breed (Sheep Genetics, 2020). Selection has been validated in Australian Merino flocks as well. Selection for lower FEC has reduced FEC in lambs and periparturient FEC rise in ewes (Woolaston et al., 1990; Woolaston, 1992).

Resistance to parasitism and prevention/elimination of infection is rooted in the immunologic response of the host. In the model of parasite resistance observed in hair sheep of Caribbean ancestry (St. Croix), prevention of adult worm establishment results in no associated

pathologies and FEC (Jacobs et al., 2015). Larval recognition and an accelerated cellular response resulting in earlier Th2 polarization occur through increased IL-4, -5 and -13 (Bowdridge et al., 2013; Bowdridge et al., 2015; MacKinnon et al., 2015; Jacobs et al., 2018). This Th2 polarization is associated with increased mucus release from goblet cells and smooth muscle contractility (Zhao et al., 2008; Horsnell et al., 2011). Consequently, an unfavorable environment exists for larval development and adult worm establishment. In parasite-susceptible breeds, delayed cellular and humoral responses result in increased adult worm burden and FEC (Bowdridge et al., 2013; Bowdridge et al., 2015).

The Katahdin is a composite breed resulting from the cross of "African Hair Sheep" imported from the Caribbean Island of St. Croix and traditional wool breeds. These 'African Hair Sheep" were likely Virgin Island Whites, early ancestors of the St. Croix (Wildeus, 1997). Michael Piel of Maine began these crosses in the late 1950's and once satisfied with the population, named them Katahdin after the tallest mountain in Maine (Breed Origin & History, 2019). Since that time, the easy-care, forage-adaptable attributes of the Katahdin have resulted in their growth into the most frequently registered breed in the United States (Morgan, 2019).

The Katahdin breed is commonly associated with parasite resistance. However, great variability exists within the breed for parasite resistance traits (Notter et al., 2007; NSIP Searchable Database, 2019). It is assumed the modern Katahdin are related to St. Croix (Blackburn et al., 2011). Still, the herd book remains open and many other breeds may constitute a given Katahdin today. Some of these breeds may be more susceptible than others. Selection based on FEC EBV has been utilized by a subset of Katahdin breeders to improve parasite resistance in the breed. However, the mechanism used to minimize infection in these parasite-resistant Katahdins is unclear.

In this study, the FEC EBV in the Katahdin breed will be further validated through a sire selection model. Based on sire FEC EBV type, the mechanism of *Hc* resistance will be evaluated. It could be hypothesized that the relatedness of St. Croix and Katahdin may result in a St. Croix-like mechanism of *Hc* immunity in which more resistant individuals are able to target larval stages, prevent adult establishment and consequently have reduced FEC.

Materials and Methods

Breeding Scheme

Details regarding the breeding scheme used to produce lambs for this study were outlined in Chapter III. Briefly, Katahdin sires with exceptionally high (High FEC) or low FEC (Low FEC) EBV were selected (Year 1 (YR1), n = 4; Year 2 (YR2), n = 8; Table 1). In YR2, one high sire and one low sire from YR1 were utilized to connect the years. The average difference in sire post-weaning FEC (PFEC) EBV between High and Low FEC sires was 300.9% in YR1 and 421.0% in YR2. Katahdin ewes at the Southwest Virginia Agricultural Research and Extension Center (SWAREC; Glade Spring, VA) were randomly assigned to service sire mating groups with equal distribution of ewe age. Mating began on October 15 each year and rams were removed after 45 days.

Management

All animal procedures were approved by the West Virginia University Animal Care and Use Committee (protocol 1608003811.1). Lambs were born mid-March to early-April and managed on pasture with their dams until 90-120 days of age. Lambs were then removed from pasture, treated with levamisole hydrochloride (8 mg/kg, Agrilabs) and transported to the West

Virginia University Animal Sciences Farm. Upon arrival, lambs were quarantined for two weeks. Lambs were housed in a covered facility bedded with sawdust or straw. Lambs did not have access to forage. Lambs were fed a complete pellet (16% CP) ad libitum with access to water. Lambs were fed for 90 days prior to infection. Lambs were randomly allocated by sire type and sex to four pens. Sexes (ewes and rams) were housed separately in alternating pens. Sire and sire type were represented by an equal number of lambs in each pen.

After the rest period, FEC was measured to confirm anthelmintic efficacy (FEC ≤ 200 eggs/g) prior to artificial infection. Lamb FEC reduction was similar between Low FEC- and High FEC-sired lambs (YR1: 74 vs. 60%; YR2: 96 vs. 98%; respectively). In YR1, two High FEC-sired lambs had greater FEC after anthelmintic treatment. These outliers were removed from reduction analysis.

Infection and Data Collection

In YR1, all (n = 113) lambs were infected with 10,000 *Hc* L3 in a single dose. The infection was allowed to persist for five weeks. In YR2, 60 lambs (30 High FEC EBV, 30 Low FEC EBV) were selected at random from the total population (n = 117) and infected with 5,000 *Hc* in a single dose due to limited L3 supply (Table 1). Sire, sire type and sex were represented by an equal number of lambs. The remaining lambs were left uninfected. The infection was allowed to persist for five weeks. During the infection period, FEC, PCV and body weights were collected weekly on all lambs.

A modified McMaster test was used to measure FEC (Whitlock, 1948). Raw egg counts were multiplied by 50 to determine FEC in eggs/g. Blood was collected via jugular venipuncture from each lamb in 5-mL tubes containing EDTA (Tyco, Mansfield, MA, USA) for packed cell

volume (PCV) measurement. Microhematocrit tubes were filled and centrifuged at 13,700 *x g* for 2 minutes (Beckman Coulter, Atlanta, GA, USA).

Harvest

At the end of the infection period, lambs (n = 60) were harvested at the Virginia Tech Meat Center. In YR1, lambs to be harvested were selected at random with sire, sire type, and sex represented by an equal number of lambs. In YR2, all infected lambs were harvested. Lambs selected for harvest were allocated randomly to one of two consecutive harvest days. Abomasal contents were flushed and adult worms collected. In YR1, total contents were collected and 200 ml of 10% formalin (Thermo Scientific, Waltham, MA, USA) added. In YR2, total abomasal contents were collected and water was used to bring contents to 2 L. A 10% aliquot was collected for measurement. An equal volume of formalin was added to the sample. Total worm count (WC) was determined. In YR2, worm counts were multiplied by 10 to reflect total worm burden. The first 100 worms randomly sampled were mounted on slides with lactophenol. Images were captured of adult worms and worm length determined using Image J software (National Institutes of Health, Bethesda, MD, USA).

Statistical Analysis

Data were analyzed using the Mixed Model procedure of SAS (SAS institute, Cary, NC). Fixed effects of sire type (High FEC, Low FEC) and time were utilized with random effects of individual sire within sire type. In YR2, sire type was evaluated within infection status as only 60 lambs were infected. All data presented represent only infected lambs. Worm count and FEC

data were normalized using a log transformation in which lnWC = ln(WC +1) and lnFEC = ln(FEC+1) for Mixed Model analysis.

Distribution data were analyzed using the Genmod procedure of SAS with fixed effects of sire type. In YR2, one Low FEC-sired lamb was removed prior to harvest due to rectal prolapse. Therefore, WC data represent 29 Low FEC-sired lambs. Sample collection for harvest FEC was limited to 25 Low FEC- and 26 High FEC-sired lambs, respectively in YR1 and 27 Low FEC- and 27 High FEC-sired lambs in YR2.

Worm fecundity was predicted based on FEC and WC by sire type and analyzed with the General Linear Model procedure of SAS. The linear regression model was:

$$Y_{FEC} = \beta_1 WC + \beta_2 Trmt + (\beta_1 WC * \beta_2 Trmt) + e_{FEC}$$

where $\beta_1 WC$ represents the worm count at harvest, $\beta_2 Trmt$ represent sire type, $(\beta_1 WC * \beta_2 Trmt)$ represents the interaction of worm count and sire type, and e_{FEC} represents the random error associated with the prediction of FEC. The relationship between PFEC EBV and WC was analyzed using the Reg procedure of SAS where linear regressions were fit for harvest FEC and WC on PFEC EBV.

Significance was determined at $P \leq 0.05$. Tendencies were determined at $0.05 < P \leq 0.10$.

Results

Infection Data

Data presented here represent lamb response to a challenge infection. All lambs were exposed to a GIN infection prior to removal from pasture and substantial FEC was observed in lambs during this time period (Chapter III).

In both years, FEC remained constant and similar between sire types for the first two weeks of the infection period (prepatent period). After this time point, FEC increased indicating the establishment of a *Hc* infection. In each year, High FEC-sired lambs had numerically greater FEC during weeks 4-6 of infection (Figure 1A, 1E). In YR1, change in FEC after the prepatent period was greater for High FEC-sired lambs ($P < 0.05$, Figure 1B). A similar trend existed in YR2. Level of anemia as indicated by PCV was similar between High and Low FEC-sired lambs. Numerically, PCV increased in Low FEC-sired lambs following the prepatent period compared to High FEC-sired lambs (Figure 1D, 1H).

Growth Performance

Lamb body weights did not differ at the start of the infection period in either year. Over the infection period, average daily gain was similar between sire types and body weight did not differ at harvest (Figure 2). Differences in lamb growth between years could be attributed to stage of maturity. In YR1, lambs were infected in July immediately following transition to ad libitum diet. In YR2, lambs were infected in late October after 90 days on ad libitum feed.

Worm Counts

Harvest FEC and abomasal WC are presented in Figure 3. At harvest, FEC was similar between sire types. In both years, worm burden was numerically greater but not significantly different (YR1, $P = 0.54$; YR2, $P = 0.16$) in High FEC-sired lambs compared to Low FEC-sired lambs. Worm length did not differ in either year (YR1, $P = 0.32$; YR2, $P = 0.35$) but was numerically greater in High FEC-sired lambs.

Distributions of harvest FEC and WC differed by sire type (Figure 3C-D). In YR1, there was a tendency for a greater number of Low FEC-sired lambs to have FEC between 101 and 500 compared to High FEC-sired lambs ($P = 0.10$). In contrast, a greater number of High FEC-sired lambs had FEC over 500 compared to Low FEC-sired lambs ($P < 0.05$). In YR2, a greater number of Low FEC-sired lambs tended to have FEC of zero compared to High FEC-sired lambs ($P = 0.09$). Additionally, a greater number of Low FEC-sired lambs had an adult WC of zero compared to High FEC-sired lambs ($P < 0.05$). A numerically greater number of High FEC-sired lambs had WC between 101 and 500 compared to Low FEC-sired lambs ($P = 0.11$). Compared to High FEC-sired lambs, a greater proportion of Low FEC-sired lambs were near zero for FEC and WC.

To better understand worm fecundity, FEC was regressed on WC (Figure 4A). In YR1, worm fecundity (FEC/WC) was greater in High FEC-sired lambs compared to Low FEC-sired lambs ($P < 0.05$). A similar trend existed in YR2 ($P = 0.08$). Regressions were used to evaluate the PFEC EBV as a predictor of WC and FEC. In general, as PFEC EBV increased, WC and FEC increased. However, these relationships were not significant. For WC, slopes were similar between YR1 and YR2. More variation existed for the relationship between PFEC EBV and FEC between years.

Discussion

The FEC EBV was introduced in 2003 (Notter et al., 2007). Since 2010, the number of Katahdin sheep enrolled in genetic evaluation programs has nearly doubled while breed average WFEC indicates progress in selection for parasite resistance (Sheep Genetics, 2020). In Chapter III, sire FEC EBV was associated with lamb FEC shortly before weaning and after 3 weeks post-weaning. In spring lambing flocks, the weaning time period coincides with primary GIN infections, which are typically more severe than subsequent challenge infections (Jacobs et al., 2015). Weaning stress may influence lamb parasitism regardless of FEC EBV.

For genetic evaluation of FEC and resistance to parasite infection, equal opportunity for infection is assumed. Yet, variation in infection status based on litter size, lamb age and dam age is documented (Notter et al., 2017). Post-weaning artificial infections better control environmental factors and ensure equal infection.

Here, lambs were removed from pasture at 90-120 days of age. Based on YR2 FEC, parasite burden existed at 45-60 days of age and rose rapidly up to 3-4 weeks post-weaning when lambs were removed from pasture (Chapter III). Significant infection during this period and opportunities for reinfection may have improved response to further infections (Lacroux et al., 2006). During the challenge infection in both years, FEC was modest (200-500 eggs/g) and similar between years despite lower L3 dosage in YR2. Even so, relative change in FEC after infection was greater in High FEC-sired lambs. Additionally, ad libitum feed intake, lamb size and age may have improved the response to infection and therefore reduced worm burden and FEC.

Despite the similarities in harvest FEC and WC averages, for Low FEC-sired lambs, these traits were not normally distributed. A greater proportion of the Low FEC-sired lambs were

able to prevent establishment and had no adult worm burden in YR2. Additionally, more extreme FEC were observed in those lambs sired by High FEC rams. Thus, similarities could be drawn between Low FEC-sired lambs that prevented infection and St. Croix resistance to *Hc*. The accelerated response to infection in St. Croix results in larval expulsion and no adult worm establishment (Bowdridge et al., 2013; Bowdridge et al., 2015; Jacobs et al., 2016). Consequently, FEC is not observed in St. Croix during challenge infection (Gamble and Zajac, 1992; Jacobs et al., 2015).

Even so, adult worms established in Low FEC-sired lambs were numerically shorter than those in High FEC-sired lambs (not significant). Worm fecundity was greater in High FEC-sired lambs compared to Low FEC-sired lambs. Thus, similarities could be drawn between Low FEC-sired lambs and Texel resistance to *Hc* when adult worm establishment is allowed. In chapter II, adult worm burden was observed, yet FEC was reduced compared to parasite-susceptible Suffolk lambs. Worm length was shorter in Texel vs. Suffolk lambs and worm fecundity was reduced. Shorter worm length has been associated with a reduction in worm fecundity (Stear et al., 1995; Rowe et al., 2008). In mouse models, *Heligmosomoides polygyrus* worm burden is B cell dependent. Worm number and length is greater in B cell deficient mice (Liu et al., 2010). These data indicate that worm burden and size may be under humoral control. In sheep, IgA is correlated negatively with worm length (Stear et al., 1995). Immunologic control of worm burden, size, and consequently fecundity may dictate parasitism based on sire EBV type.

The FEC EBV provides a tool by which progress can be made in reducing effects of parasitism through selection (h^2 = 0.18-0.23; Ngere et al., 2018) with little antagonism to growth traits. This heritability is similar to that observed for periparturient FEC rise (Notter et al., 2018) and for birth, weaning and post-weaning weights (Ngere et al., 2017). Previous data have

indicated small genetic correlations between FEC EBV and weight traits and phenotypic correlations near zero. Even so, WFEC and PFEC were highly related (Ngere et al., 2018).

Here, correlations were small for FEC and WC of lambs and PFEC EBV. However, the level of infection was modest, as mentioned previously. Additionally, lambs previously had been exposed to significant infections reported in chapter III. Thus, even High FEC-sired lambs may have improved immunity to *Hc* based on past exposure and maturity.

In summary, selection for improved parasite resistance based on the FEC EBV will result in a corresponding reduction in FEC change during a challenge infection. Further, selection should be independent of other production traits such as growth. By selecting for reduced FEC in Katahdin sheep, worm establishment and fecundity were impaired. A subset of individuals limited adult worm establishment and therefore do not have a FEC in a St. Croix-like manner. Those established adult worms were limited in their reproductive prolificacy. Taken together, the FEC EBV is an effective measure to mediate worm burden and FEC. Variability resulting from the composite structure of the breed may influence individual variation in immunological response to *Hc*.

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Tables and Figures

Table 1. Sire summary for Year 1 (YR1) and Year 2 (YR2) with sire estimated breeding values (EBV).

				EBV[4]	
Sire ID[1]	YR1 Lambs[2]	YR2 Lambs[2]	YR2 Infection[3]	PWWT (kg)	PFEC (%)
Low Sire 1	31 (13/18)	14 (7/7)	8 (4/4)	6.0	-67.8
Low Sire 2	26 (16/10)	-	-	3.1	-81.5
Low Sire 3	-	15 (7/8)	8 (4/4)	4.6	-99.5
Low Sire 4	-	14 (8/5)	7 (4/3)	-1.1	-99.1
Low Sire 5	-	14 (6/8)	6 (3/3[*])	2.0	-78.7
Low FEC Average	**57 (29/28)**	**56 (28/28)**	**29 (15/11)**	**2.9**	**-85.3**
High Sire 1	22 (12/10)	14 (7/7)	8 (4/4)	1.3	348.8
High Sire 2	30 (15/15)	-	-	3.7	103.5
High Sire 3	-	15 (8/7)	7 (3/4)	3.6	509.7
High Sire 4	-	14 (7/7)	7 (4/3)	2.4	120.4
High Sire 5	-	15 (8/7)	8 (4/4)	1.7	359.8
High FEC Average	**52 (27/25)**	**58 (30/28)**	**30 (15/15)**	**2.5**	**288.4**
Totals	**109 (56/53)**	**114 (58/56)**	**59 (30/26)**		

[1]Sire ID denotes grouping based on FEC EBV. Two sires in each group were utilized for breedings in YR1. Three new sires along with a carryover sire were used in YR2.

[2]Represents number of lambs (male/female) in each year by sire.

[3]Number of lambs (male/female) infected in YR2. A subset of lambs were infected and the rest remained uninfected.

[4]Sire EBV based on 6/15/2019 Lambplan analysis; post-weaning weight (PWWT) and post-weaning fecal egg count (PFEC).

[*]Four female lambs were infected but due to rectal prolapse one was removed from analysis

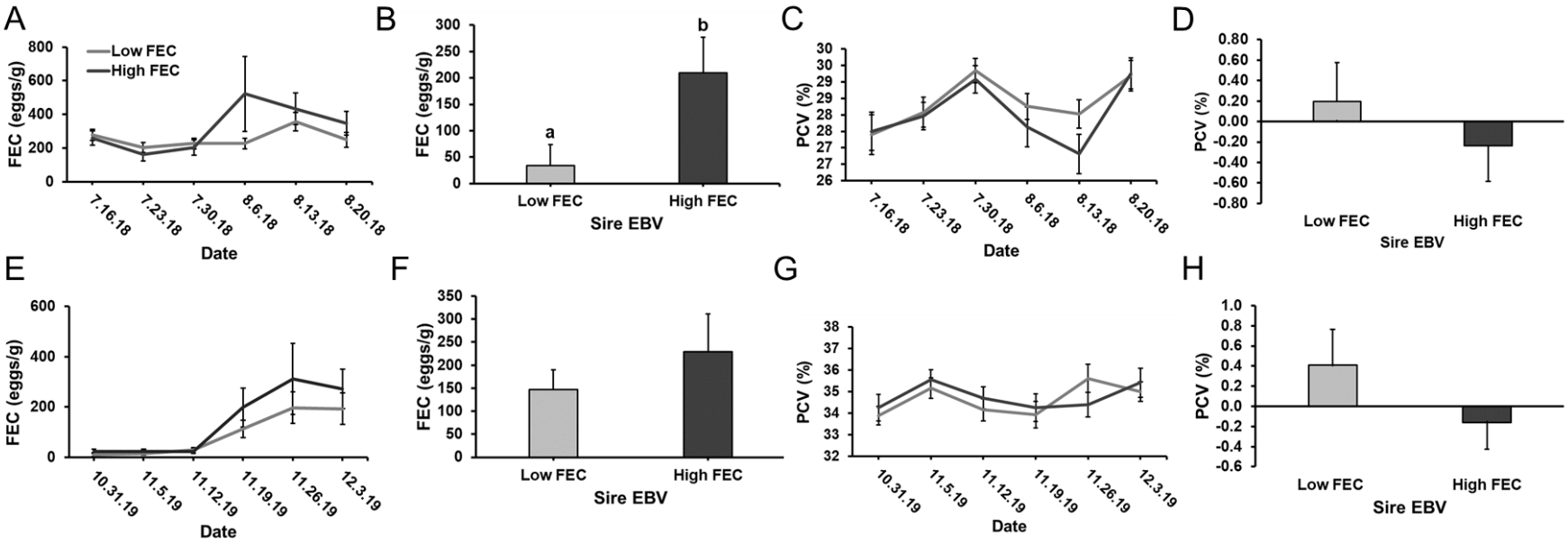

Figure 1. Challenge infection performance. Lamb fecal egg count (FEC, A-B, E-F) and packed cell volume (PCV, C-D, G-H) during infection with *Haemonchus contortus*. (B,F) Change in FEC after the prepatent period (weeks 0-2). (D,H) Change in PCV after the prepatent period. Lambs were infected on 7/16/18 (Year 1) with 10,000 *H. contortus* L3. Lambs were infected on 10/31/19 (Year 2) with 5,000 *H. contortus* L3. Graphs A-D represent Year 1. Graphs E-H represent Year 2. Low FEC and High FEC designate sire EBV type. Error bars represent standard error of the mean (SEM). Different letters denote significance $P \leq 0.05$. Time was significant in A,C,E, and G ($P \leq 0.05$).

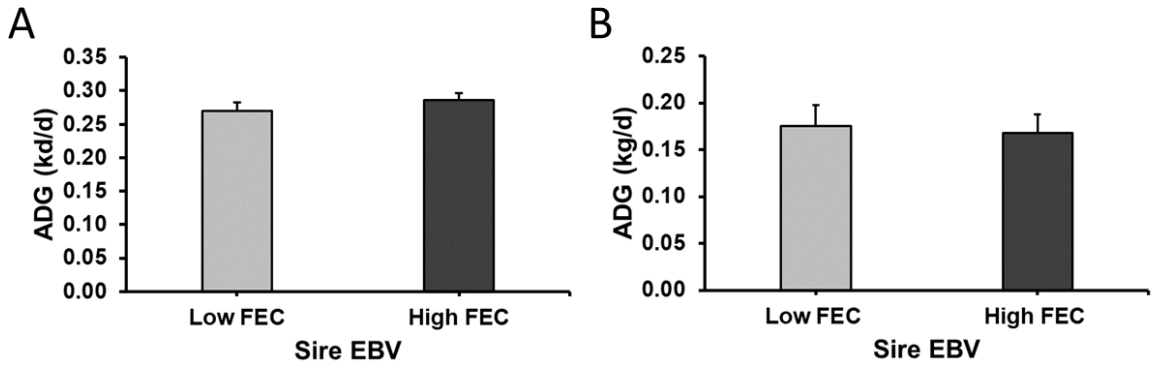

Figure 2. Growth performance. Average daily gain (ADG) during infection with *Haemonchus contortus*. Lambs were infected on 7/16/18 (Year 1, A) with 10,000 *H. contortus* L3. Lambs were infected on 10/31/19 (Year 2, B) with 5,000 *H. contortus* L3. Graphs A-C represent Year 1. Graphs D-F represent Year 2. Low FEC and High FEC designate sire EBV type. Error bars represent standard error of the mean (SEM).

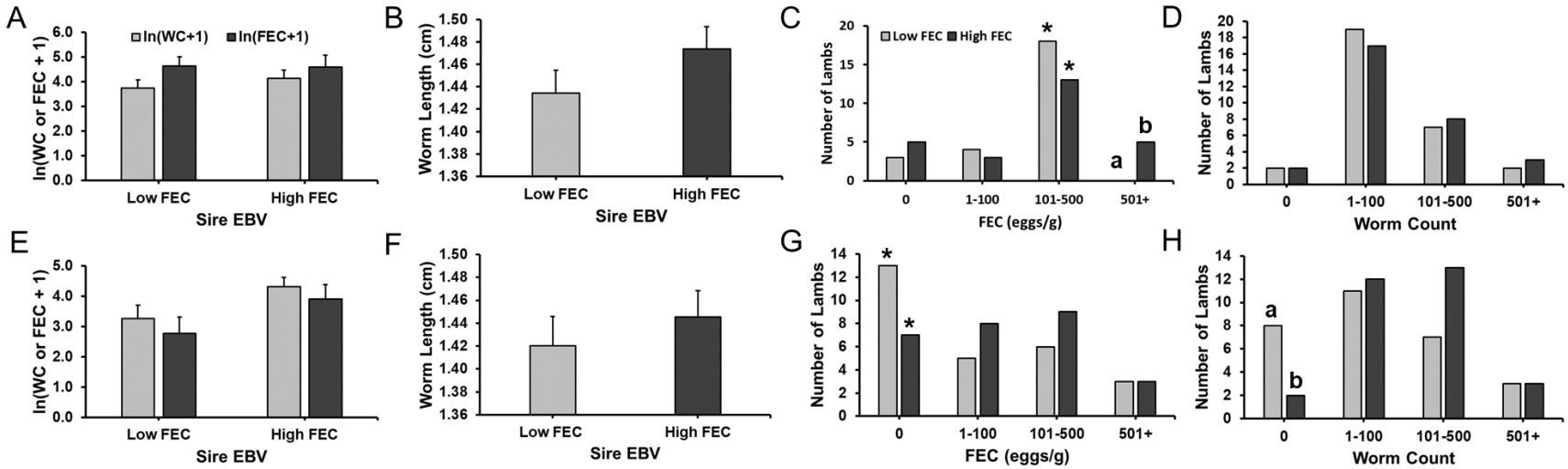

Figure 3. Adult worm counts and harvest fecal egg count (FEC). Lambs were harvested in two consecutive days after the last sample collection (Figure 1). Graphs A-D represent Year 1. Graphs E-H represent Year 2. Adult worm count and FEC at harvest were log transformed for normality (A and E). Adult worms were fixed on slides and length (B and F) measured using Image J. Fecal egg count (FEC; C and G) and worm count (D and H) distribution by sire type. Different letters denote significant $P \leq 0.05$. Tendencies denoted by * $0.05 < P \leq 0.10$.

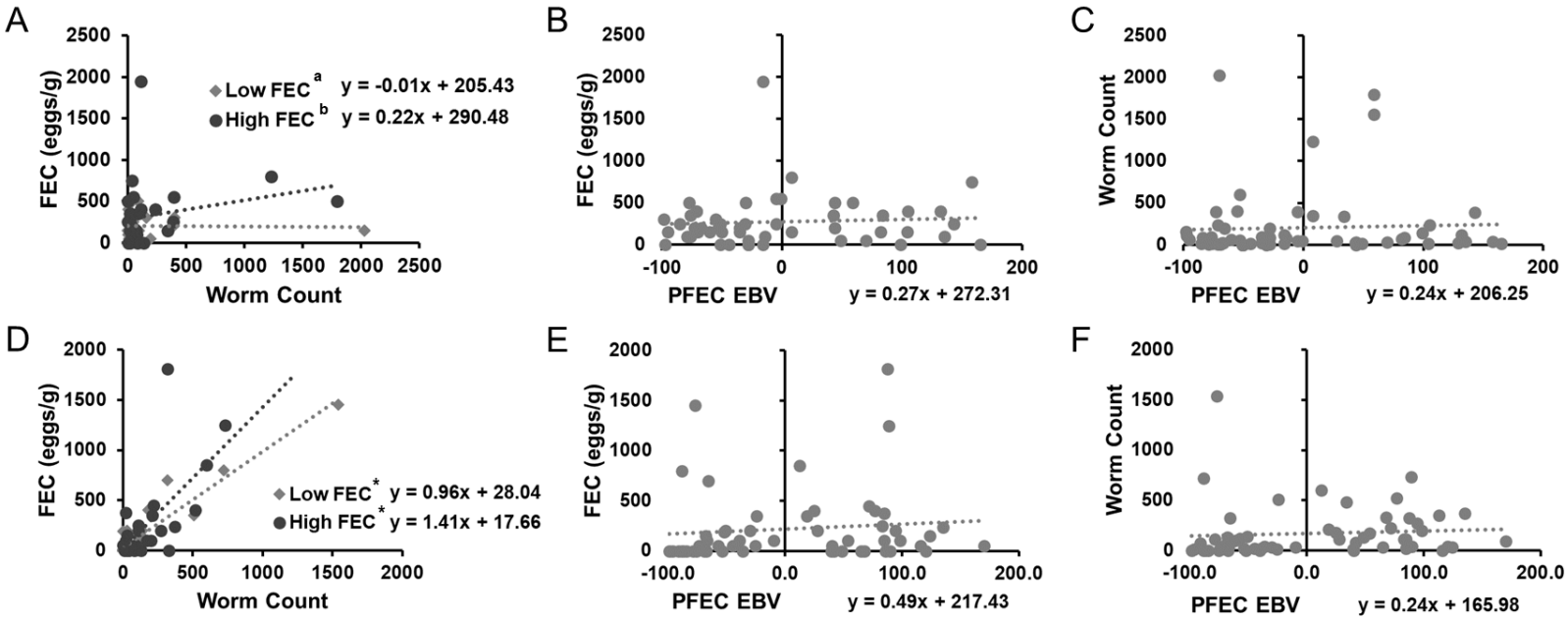

Figure 4. Relationships between worm count, fecal egg count (FEC) and post-weaning fecal egg count (PFEC) estimated breeding value (EBV). Graphs A-C represent Year 1. Graphs D-F represent Year 2. Regressions of FEC and worm count (A and D) by sire type. Relationship between FEC (B and E) or worm count (C and F) and lamb PFEC EBV across sire types. Different letters denote significant $P \leq 0.05$. Tendencies denoted by * $0.05 < P \leq 0.10$.

CHAPTER V: DISCUSSION AND FUTURE DIRECTIONS

Introduction

Production losses from anthelmintic resistant gastrointestinal nematode (GIN) infections have resulted in establishment of integrated parasite management plans. These management systems include selective anthelmintic treatment, intensive grazing systems, nutritional supplementation, anthelmintic alternatives such as copper oxide wire particles, and genetic selection. The fecal egg count (FEC) estimated breeding value (EBV) is a metric utilized for quantitative selection to improve parasite resistance through reduction in FEC. The Katahdin breed has the most robust FEC dataset in the National Sheep Improvement Program and progress from selection has been successful (Sheep Genetics, 2020). Even so, many breeds, such as Texel, have a limited or nonexistent FEC dataset. Regardless, even sheep bred for enhanced resistance are lost to parasitism under stressful environmental conditions. Thus, an integrated approach is necessary. Here, implications of the previous findings will be discussed with possible direction for future studies.

Selection Summary

Variation in resistance to GIN exists within and among breeds (Gamble and Zajac, 1992; Notter et al., 2007; NSIP Searchable Database, 2019). Parasite resistance models have relied on variation among breeds. St. Croix hair sheep and Suffolk (Jacobs et al., 2015) or Dorset (Gamble and Zajac, 1992) have served as parasite-resistant and parasite-susceptible models, respectively. These breeds have allowed for characterization of resistance mechanisms and illustrate genetically distinct breeds (Blackburn et al., 2011). The practicality of St. Croix, despite their

resistant phenotype, is limited due to their lack of market acceptability. Nonetheless, FEC EBV is now available from some Suffolk and Dorset breeders making selection for improved resistance in these traditionally susceptible, but more marketable, breeds possible.

Parasitology labs processing fecal samples and providing FEC data for genetic evaluations are limited. Without those resources, generation of FEC data will not be possible in the future. The FEC EBV is currently the most valuable tool to evaluate parasite resistance within breed. Efforts have been made to introduce a FAMACHA™ score EBV. However, given the categorical and subjective nature of the trait, accuracy of selection and opportunity for progress may be hindered compared to selection based on FEC EBV. Historically, parasitology labs generating these data are overseen by veterinary parasitologists primarily concerned with diagnostics. However, FEC used solely for genetic evaluation do not need veterinary oversight. Thus, other small ruminant labs should consider processing fecal samples for sheep producers with a specific interest in generating FEC EBV within their flock.

Texel Summary

Texel sheep were introduced to North America in 1985 (Leymaster and Jenkins, 1993). The Texel originated from the Netherlands and has become popular across Europe as the elite terminal sire breed. Texel rams comprise 27% of all sires used in the U.K. (Year in Review, 2020). Yet, utilization in U.S. breeding schemes is still limited.

The sheep business in the Eastern U.S. centers around the non-traditional or ethnic market. This market desires a moderate sized lamb weighing 30-50 kg (Shiflett et al., 2010). In this region, lamb is typically forage-finished with significant parasite challenges. Given evidence in Europe (Good et al., 2006) as well as here, Texel sheep should be branded as the elite parasite-

resistant terminal sire breed for the Eastern U.S. sheep market. The moderate mature size, early maturity, improved carcass composition, and parasite resistance make Texel adaptable to forage-based production systems, marketable to non-traditional buyers, and complementary to growing hair sheep ewe flocks. Not to mention, in graded-markets, Texel genetics have improved lamb grade and value.

The potential for Texel sheep is immense. Even so, a few concerns should be addressed to best integrate Texel into selection schemes and management programs. First, involvement of Texel producers in NSIP is limited. In 2019, there were 111 lambs entered into the genetic evaluation for Texels. Compare that to Katahdin at 5,308 or Australian Merino at 80,000+ and Texels have significant room for growth. Fecal egg count data are even more limiting with only one Texel producer submitting data in 2019. Significant within breed variability exists for FEC EBV in Katahdins (NSIP Searchable Database, 2019). Variation in sire FEC EBV has significant impacts on progeny FEC, mechanisms of resistance and lamb fitness. Similar variability could be assumed for all breeds. Without FEC EBV data, branding all Texels as parasite-resistant becomes a question of conscience.

Increased NSIP participation in all breeds, particularly Texel, is needed for a greater understanding of the genetics underpinning parasite resistance. Development of extension programs to aid producers in data collection and submission may be necessary. Additionally, changing selection mindsets away from the showring toward quantitative tools (EBV) is the only way needs of the commercial producer will be met by seedstock breeders. However, this will remain difficult until value is proven for performance-oriented selection. That value will need to come through direct marketing programs, value-based pricing and performance of seedstock genetics in commercial environments. One example could be the development of marketing

cooperatives that sell terminal-sired lambs that have weight and management specifications requiring the utilization of Texel sires with given EBV for growth and parasite resistance. If the value added to the lamb crop due to enhanced performance can be reinvested in seedstock, then seedstock producers may be more willing to invest in selection tools.

To address seasonality in the sheep industry, out-of-season breeding and accelerated lambing programs have been implemented by producers. These systems require breeding during typical seasons of ewe anestrus and ram infertility (Lewis et al., 1996; Karagiannidis et al., 2000; Vincent et al., 2000). Breeds such as Dorset, Polypay, and Katahdin have been integrated effectively into these programs. Utilizing genetic stock known to be reproductively active during late spring and summer months improves success of these systems. Thus, incorporation of terminal sire breeds not traditionally selected for aseasonality (i.e. Texel) could be challenging. Substandard fertility in Texel rams during summer breeding could limit pregnancy rates even if the maternally-bred ewe flock is cycling. One solution could be composite rams. F_1 Texel crosses would combine the muscularity of the Texel with improved fertility while maintaining fitness traits such as parasite resistance. Dorset could be a logical option. The dual-purpose Dorset, also known as "the breed for all seasons," offers less seasonality while maintaining the muscling traits expected in terminal sires. Additionally, FEC EBV on Dorset sheep could allow selection of more parasite-resistant individuals to include in composites. Polypay and Katahdin genetics should be reserved for maternal purposes. Lack of selection for muscling in these breeds would make F_1 terminal sires average, forfeiting opportunities for progeny premiums based on lamb composition. Not to mention, Katahdin X Texel hybrids would require shearing of an inferior, unmarketable fleece. Another solution could be reservation of terminal sires like Texel

for traditional fall breeding. Purebred out-of-season production could be utilized for generation of replacements ensuring perpetuation of aseasonal genetics.

Hybrid rams should be evaluated for aseasonal libido and market lamb production. For proof of concept, purebred Texel, Dorset and F_1 Texel X Dorset rams should be randomly mated to a commercial ewe flock beginning May 1. Ram exposure should last 34 days. Lambing will begin in early October and pregnancy rate determined based on the number of ewes lambed per ewe exposed for each sire breed. Progeny should be fed out on fall stockpiled forage and fed as necessary through the early winter until lambs are approximately 40 kg. Carcass ultrasound should be used for ribeye area and fat measurements. Graded auctions will be used for lamb value determination. Based on sale prices, premiums based on sire breed can be determined as well as lamb value per ewe exposed as a metric of fertility, lamb growth and composition.

Given the mechanism of parasite resistance in Texel sheep, maintenance of adult worm burden within the flock cannot be ignored. In the case of *Hc* infections, FAMACHA™ scoring should be utilized regularly to assess parasitism in ewes and lambs and provide treatment as necessary. In times of increased stress, failure of the immune system to mitigate adult worm fecundity could result in increased FEC. This could include the periparturient period, post-weaning period, transportation stress or stress induced by secondary bacterial infections. Nonetheless, over time, reduced FEC output should result in lower pasture contamination and opportunities for reinfection.

To evaluate Texel susceptibility to stress and consequent effects on FEC, a series of studies should be conducted on artificially infected Texel lambs. Stressors examined should include lipopolysaccharide challenge and simulation of long-distance transportation (10-12 hours). Lambs should be infected and the infection allowed to persist for 4 weeks. At this time,

the stressor should be applied and the infection monitored by FEC for an additional 3 weeks. Lambs should be harvested at the end of the study and adult worm burden determined. If true adult worm-mediated immunity exists, little to no FEC should be detectible prior to stress even though adult worm burden may be present. If the stressor does impact adult worm-oriented immunity, FEC should rise after the stressor is applied. Adult worm burden should be used to confirm establishment in both stressed and control groups.

Great potential exists for Texel sheep in the eastern U.S. Market suitability and adaptability to forage/hair sheep-based production should classify the breed as the elite terminal sire for this region. However, further work is needed to identify genetic variability in parasite resistance, adaptability to aseasonal breeding, and susceptibility to environmental stressors.

Katahdin Summary

The composite nature of Katahdins has resulted in significant within breed variability for FEC. Variation based on the FEC EBV ranges from -100 to 1800%, or a 1900% change in genetic merit for post-weaning FEC between the most resistant and most susceptible individuals within the breed (NSIP Searchable Database, 2019). Thus, one could hypothesize that parasite resistance observed in Katahdin could result from genetic similarity with St. Croix (Blackburn et al., 2011). However, based on data presented in Chapter IV, potentially only a small fraction of individuals within Katahdin have a parasite-resistant phenotype characterized by a lack of adult worm infection and no FEC. Other individuals become highly parasitized and some are intermediate. Thus, the mechanism of resistance is variable, and more work is needed to understand how this variation relates to magnitude of FEC EBV. Given the random mating scheme in these studies, only 7% of lambs across both years had PFEC EBV below -90%.

Divergent selection of both sire and dam to generate more extreme progeny could elucidate the mechanism used by extreme negative lambs (PFEC EBV < -90%) compared to the extreme positive lambs (PFEC EBV > +150%). In the studies here, PFEC EBV for a majority of lambs was around breed average (-50% ± 20%).

Possibly more meaningful than validation of the FEC EBV or elucidation of a mechanism for resistance is the relationship between FEC EBV and lamb fitness. If FEC EBV is an indicator of general immunity, then selection for FEC EBV could be a selection tool for resistance to a variety of pathogens. Thus, selection for general disease resistance could be possible. The FEC EBV could become the primary metric for evaluation of lamb fitness. Much like the Immunity+® index in Canadian Holstein cattle measures cellular- and antibody-mediated immune responses as a metric of general disease resistance (Thompson-Crispi et al., 2012), the FEC EBV in sheep could serve a similar purpose. This would be a novel tool relevant to not only forage-based producers but also confinement operations.

Selection for parasite resistance based on the FEC EBV allows greater predictability of lamb survival regardless of environmental challenges. In Low FEC-sired lambs, lamb loss was equivalent in years with *Clostridium perfringens* type A challenges and those years without (Chapter III). In both years, losses were lower than in lambs sired by High FEC EBV rams. The FEC EBV is a tool to instill confidence and manage risk.

The primary selection index utilized by Katahdin breeders is the USA Hair Index comprised of EBV for weaning weight, maternal weaning weight, number of lambs born and number of lambs weaned. This is an economic index with traits weighted based on their value per one unit increase in the given trait, all others held constant. Consideration should be given to incorporating the FEC EBV into this metric. Assessing the economic value of FEC has been

challenging. Even so, the association of FEC EBV with lamb survival and the cost of associated death loss could be a place to begin. Regardless, the FEC EBV could be included in a lamb pasture performance index along with weight traits. Traits in this index could be weighted based on perceived importance to sustainable pasture-based lamb production and not be economically weighted. Further discussion with producer groups could help elucidate relative weights given to these traits.

The FEC EBV could be especially relevant in management systems that are not necessarily challenged by parasitism. Other pathogens, such those causing respiratory and digestive diseases, may be more prevalent in confinement systems but are much more difficult to quantitatively assess.

Further evaluation of the FEC EBV should consider relationships with bacterial diseases (clostridial, respiratory, mastitis, foot rot, etc.) across breeds and environment. With the largest dataset, Katahdin may serve as a foundation breed for this work. Nonetheless, integration of FEC EBV and selection for disease resistance into Midwest and western range flock could have significant impacts on traditional lamb production. Given minimal parasite challenge in some of these environments, data collection from a subset of individuals within the breed could be used to generate FEC EBV and pedigree relationships used to apply the EBV across flocks. Thus, selection for disease resistance and lamb fitness in both the range as well as confinement/feedlot environments may be possible. Reduction in lamb losses from clostridial and respiratory challenges and improvement in ewe longevity through mastitis prevention could have monumental impacts on productivity.

Divergently selected lambs could be evaluated in these feedlot environments and health status monitored. Continuous temperature loggers would provide an additional metric of

subclinical infection status. Association of FEC EBV with body temperature, antibiotic treatment frequency, and death loss would further verify the relationship of FEC EBV, disease fitness and general immunity. Not to mention, costs associated with health-related treatment and losses could be used to better understand the value of FEC EBV. Collaboration with large flocks measuring mastitis frequency with detailed culling records that have FEC data could begin elucidating relationships between FEC EBV, occurrence of mastitis and ewe longevity. The impact of FEC EBV selection on lamb and ewe performance and health status could have substantial impacts on economic sustainability for producers across breeds and regions.

Management Alternatives

Genetic selection alone may not be a sufficient solution for anthelmintic resistance in worm populations. Given environments with severe parasite burden and lack of anthelmintic efficacy, alternatives must be considered to sustain small ruminant production. One solution to parasitism and associated losses is removal of sheep from infected environments. Nutritional supplementation in a dry lot environment where reinfection cannot occur would provide a more favorable environment for expression of genetic potential in other economically relevant traits. This could be particularly beneficial to individuals with greater susceptibility to parasitism such as young lambs and those lambs born to younger ewes in larger litter sizes (Notter et al., 2017). The timing of pasture removal as well as the economic factors associated with confinement management should be considered.

Based on data presented in chapter III and observed in the transition period prior to infection, lamb performance is compromised by post-weaning stress and the associated increase in parasite burden. Once removed from pasture and parasite burden cleared, lamb growth is

compensatory for 45-60 days. Lamb growth during this period was close to 0.45 kg/d compared to gains of approximately 0.20 kg/d during the rest of the feeding period. Improved gains during this period could result in marketable lambs at young ages and heavier weights than traditionally achieved on pasture. Additionally, breeding success of ewe lambs could be improved. Removal of lambs from pasture at weaning may compensate for increased susceptibility to parasitism during the early post-weaning period.

Development of protective immunity to GIN in replacement breeding stock should be an important consideration in management systems that combine pasture utilization and confinement feeding. Development of lambs on pasture prior to weaning would allow for parasite exposure, quantification of parasite burden by FEC and development of antigen recognition while lactation by the dam supplements lamb performance. Once weaning occurs, lambs could be housed in a dry lot for 45-60 days and then market lambs sold and replacement ewe lambs returned to pasture. These ewe lambs would be larger, more mature and better capable of handling parasite infection. In addition, they would have some level of adaptive immunity developed from the pre-weaning primary infection. This could be one opportunity to optimize parasite-resistant genetics with improved management during times of increased susceptibility.

The solution to parasitism and the optimization of production efficiency rests in no single management tool. Rather, producers should examine the full toolbox of resources available and develop integrated systems to manage production challenges. Genetic selection is just one tool in the toolbox. Nonetheless, significant progress is possible through selection. As Jay Lush described in Animal Breeding Plans, “the genes cannot develop the characteristic unless they have the proper environment, and no amount of attention to the environment will cause the

characteristic to develop unless the necessary genes are present." He continued by illustrating the greater magnitude of within breed variability compared to variability among breeds (Lush, 1943). This has also been supported in more recent analyses (Lawson Handley et al., 2007; Blackburn et al., 2011). Thus, accurate identification and selection of sires within breed is critical to genetic progress. Realization of genetic progress is only possible with the proper environment. Moving forward, integration of genetic and environmental tools will be essential to management of anthelmintic resistant helminth populations and sustainability of small ruminant production.

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